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Research brief

Oral creatine supplementation in humans does not elevate urinary excretion of the carcinogen *N*-nitrososarcosine

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Abstract

Objective: Creatine is a popular oral supplement in athletes and may have therapeutical potential in neuromuscular diseases. It has been hypothesized that creatine ingestion can lead to increased formation of the carcinogen *N*-nitrososarcosine.
Methods: We investigated in a double-blind, placebo-controlled study the urinary excretion of *N*-nitrososarcosine after 1-wk high-dose (20 g/d) and 20-wk low-dose (5 g/d) creatine supplementation in healthy humans.
Results and conclusion: Creatine ingestion does not systematically increase urinary *N*-nitrososarcosine excretion.

Keywords: Nutritional supplement; Nitrite; Cancer

Introduction

Daily oral ingestion of 5 to 20 g of creatine monohydrate, a popular food supplement, can increase creatine and phosphocreatine concentrations in muscular and neuronal tissues [1]. Creatine supplementation has been shown to enhance performance in athletes and is currently being investigated in the treatment of various neuromuscular disorders [2,3]. A concern was recently raised in *Nutrition* about the safety of creatine ingestion in humans [4]. The toxicologic problem raised refers to the fact that conditions that exist in the human stomach (i.e., low pH and availability of nitrite) are optimal to facilitate the formation of *N*-nitrososarcosine (NSAR) by nitrosation of creatine [5]. There is substantial evidence from studies in experimental animals that NSAR can act as a carcinogenic agent (liver, nasal cavity, and esophageal cancers) [6]. Further, in one study, human esophageal cancer mortality rates were positively correlated with urinary NSAR levels [7]. Thus far, the suggestion that oral creatine ingestion could be toxic is purely hypothetical because the effect of oral creatine supplementation on NSAR formation in humans is unknown. Therefore, we investigated urinary NSAR excretion during acute and long-term creatine intakes in humans.

Materials and Methods

Sixteen young volunteers (age 18.8 ± 0.3 y, 12 men and four women) gave written informed consent to participate in this study. The protocol was approved by the local ethics committee (U.Z. Gasthuisberg, Leuven, Belgium). Subjects reported that they had taken no creatine supplements for 6 mo before the study. A double-blind study was performed over a 20-wk period, during which subjects ingested placebo (maltodextrin) or creatine (creatine monohydrate). Subjects ingested 20 g of supplement daily (divided over four portions, to be taken at regular intervals throughout the day) during the first week and one 5-g dose each morning for the next 19 wk. Subjects were instructed to collect all urine during 24 h at baseline and after 1 and 20 wk of supplementation. Total urine volume was measured and

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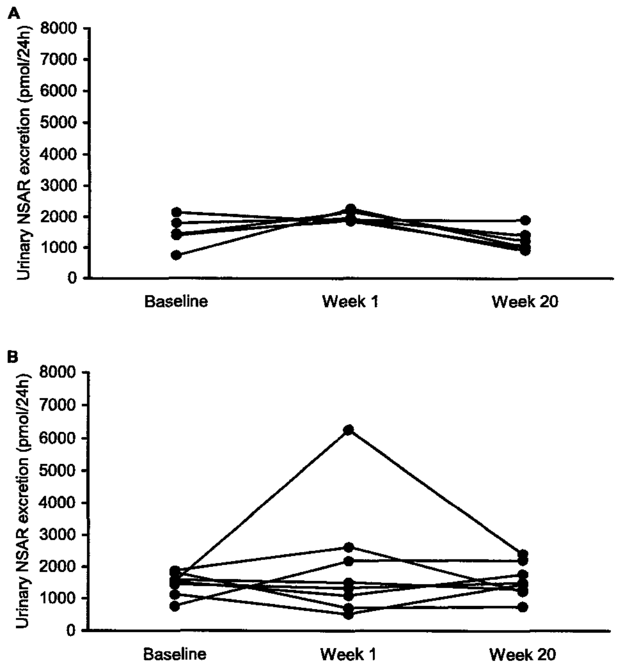

Fig. 1. Urinary NSAR excretion (picomoles per 24 h) at baseline and after 1 and 20 wk of placebo (A) or creatine supplementation (B). Data from one subject in the placebo group are not shown in A because his value at week 20 exceeded the mean ± three standard deviations. His values were 1017, 1638, and 20 663 at baseline, week 1, and week 20, respectively. NSAR, *N*-nitrososarcosine.

NSAR concentration was determined as described previously [8], with the following modifications: 2 mL of urine was assayed and, for analysis, a 15-m × 0.25 mm (inner diameter), 0.25-μm film thickness Stabilwax capillary column (Restek, Bellefonte, PA, USA) was used.

Results

Urinary NSAR excretion is shown in Fig. 1. Means ± standard errors of the mean for NSAR excretion by the placebo and creatine groups were 1416 ± 464 and 1456 ± 363 pmol/24 h at baseline, 1958 ± 227 and 2023 ± 1853 pmol/24 h after 1 wk (20 g/d), and 4018 ± 7347 and 1571 ± 541 pmol/24 h after 20 wk (5 g/d), respectively. Results indicated no significant increase in urinary NSAR in creatine-supplemented compared with placebo-treated subjects after acute high-dose or long-term low-dose ingestion.

Discussion

The present findings show that there is no significant increase in urinary NSAR excretion in humans who ingest daily supplements of 5 to 20 g of creatine monohydrate. This implies that the availability of creatine does not result in the formation of NSAR in the gastrointestinal tract, as previously hypothesized [4], although we cannot exclude the possibility that NSAR formation occurs from creatine under conditions of high nitrite concentration in the stomach. Likewise, there is no significant evidence to suspect that the popular nutritional supplement creatine is carcinogenic through formation of NSAR. Several side effects of creatine supplementation have been identified, such as increased body weight and decreased endogenous creatine synthesis [2,9]. A possible link between creatine supplementation and cancer has been suggested but is not supported by scientific or anecdotal evidence.

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Creatine Supplementation Increases Renal Disease Progression in Han:SPRD-*cy* Rats

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• The growing use of creatine as a potential ergogenic aid among active individuals has raised concern regarding its effects on the kidney, particularly among those individuals with compromised renal function. The object of this study is to investigate the effects of oral creatine supplementation in an accepted animal model of renal cystic disease. Han:Sprague-Dawley (SPRD)-*cy* rats with cystic kidney disease were administered a creatine supplement at a loading dose of 2.0 g/kg of diet for 1 week, followed by 5 weeks during which the dose was one fifth this amount, mimicking typical human consumption on a body-weight basis. Cystic kidney disease progression was assessed by measuring kidney size and fluid content and determining cyst scores. Renal function was assessed by measuring serum urea and creatinine concentrations and creatinine clearance. Creatine supplementation resulted in greater cyst growth and worsened renal function in the Han:SPRD-*cy* rat, evidenced by greater kidney weights (2.87 ± 0.08 versus 2.61 ± 0.09 g/100 g of body weight; *P* = 0.0365), renal fluid contents (89.22 ± 0.41 versus 87.38 ± 0.48 g/100 g of kidney weight; *P* = 0.0057), cyst scores (0.49 ± 0.02 versus 0.40 ± 0.03; *P* = 0.0167) and serum urea concentrations (23.96 ± 0.92 versus 20.65 ± 1.06 mmol/L; *P* = 0.0230), and lower creatinine clearances (0.125 ± 0.098 versus 0.162 ± 0.011 mL/min/100 g of body weight; *P* = 0.0159). These results indicate that creatine supplements may exacerbate disease progression in an animal model of cystic renal disease. Although systematic research of the effects of creatine supplementation in humans with compromised renal function is awaited, it follows that creatine should be used with particular caution in individuals with or at risk for renal disease.

• INDEX WORDS: Creatine; dietary supplement; ergogenic aid; kidney disease; polycystic; renal function.

Editorial, p. 157

THE POPULARITY OF oral supplementation of creatine has increased considerably since 1992, when it was observed that a significant increase in skeletal muscle concentration of this compound occurred with oral ingestion.[1] Creatine supplementation has been shown to enhance both body composition and performance during short high-intensity bouts of exercise (for reviews, see[2-6]). In addition, recent studies indicate that creatine supplementation also may have beneficial effects on lipid profiles,[7,8] neurodegenerative disorders,[9-12] and musculoskeletal abnormalities.[13,14]

More than 95% of total-body creatine is stored in skeletal muscle, where it serves as an adenosine triphosphate buffer.[2-6] Approximately one third is stored as creatine, with the remainder existing in a very high-energy phosphorylated form, creatine phosphate. On transfer of its phosphate group to adenosine diphosphate to reform the high-energy compound, adenosine triphosphate, creatine is converted to creatinine, transported from the muscle into the blood, and cleared by the kidneys.

Opposing views exist about whether oral supplementation of creatine is capable of causing a detrimental effect on the kidneys and their function.[15-21] The principal basis behind this controversy revolves around the small number of studies examining the safety of creatine supplements, the brief durations of such studies (as little as 5 days), and the characteristics of the participants (young, healthy, active men are the predominant subjects). No controlled long-term studies examining the effects of creatine supplementation on renal function have been performed because widespread consumption of this compound is a recent phenomenon. A recent

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study reported that renal function was not altered in healthy athletes consuming creatine for an average of 2.7 years.[18] However, an earlier study reported that 8 weeks of creatine supplementation was associated with greater serum urea levels.[7] In addition, two case reports suggested that creatine supplements could seriously damage the kidney.[15,17]

Because it is estimated that 1 in 500 to 1 in 1,000 individuals have inherited cystic kidney disease and many more are at risk for other renal diseases, further investigation is needed to explore potential detrimental effects of creatine supplementation on the kidney. To shed light on the potential long-term effects of creatine supplementation on renal function, we investigated the effect of oral creatine supplementation in a rat model of renal disease. The Han:Sprague-Dawley (SPRD)-*cy* rat is a well-documented and accepted animal model of inherited renal cystic disease that resembles human autosomal dominant polycystic kidney disease.[22,23] We reasoned that if creatine supplementation affects the kidney in any way, these alterations would be more readily detected in an animal model that has a shorter life span than humans and likely particularly noticeable in the Han:SPRD-*cy* rat because of the presence of a renal disorder in this model. The results of this study have implications for those with and at risk for renal disease and those for whom creatine supplementation may have other beneficial effects.

MATERIALS AND METHODS

Animals

Han:SPRD-*cy* rats were obtained from our colony, which was derived from breeding stock provided by Dr B.D. Cowley (University of Kansas Medical Center, Kansas City, KS). The experimental protocol was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals[24] and was approved by the University Animal Care and Use Committee. Animals were allowed free access to tap water and housed in individual cages with contact bedding. A 12-hour light/dark cycle was used, and the temperature of the animal facility was maintained at 22°C to 24°C, with a relative humidity of 50% to 70%.

Study Design

Four-week-old male and female Han:SPRD-*cy* rats were fed either the standard (control) American Institute of Nutrition-93G purified diet for laboratory rodents[25] or the control diet supplemented with creatine for 6 weeks. In this animal model, it is not possible preoperatively to distinguish between normal and affected animals at 4 weeks of age (beginning of study). Random assignment of 15 males and 15 females to each group resulted in the distribution of 9 diseased animals in the control male group, 14 diseased animals in the supplemented male group, and 12 diseased animals in both the control and supplemented female groups. The remainder in each group were normal. These numbers were determined by gross examination of the kidneys when the animals were killed, and only animals affected with the disease were analyzed further.

Creatine was provided as a creatine/glutamine (5:1, w/w) mixture (Crea-glutide A-DS; provided by Apex Fitness Inc, Thousand Oaks, CA). The supplement was added along with other diet ingredients at the time of diet preparation at a level of 2.4 g/kg (2.0 g/kg of creatine) of diet for the first 7 days. For the remaining 35 days, the dose for the experimental group was one fifth of this level (0.48 g/kg [0.40 g/kg of creatine] of diet). Based on food intake data and body weights of these rapidly growing animals (average values known from previous studies and confirmed at the end of this study), the daily creatine dosage was approximately 0.30 g/kg of body weight during the loading phase (week 1) and 0.03 to 0.05 g/kg in the remaining weeks of the study. This level mimics the typical supplemented human intake level on a body-weight basis (~21 g/d loading dose and ~3 g/d maintenance dose for a 70-kg man; ~17 g/d loading dose and ~ 2 g/d maintenance dose for a 55-kg woman).

Laboratory Analyses

Forty-eight hours before the animals were killed, 24-hour food and water intake measurements and urine collections were performed by means of metabolic cages. The volume of urine was determined, and an aliquot was stored at –80°C until analysis. At the end of the 6-week experimental period (at 10 weeks of age), the rats were briefly anesthetized with carbon dioxide and decapitated, and trunk blood was collected. Serum was obtained and stored at –80°C until analysis, and kidneys and livers were removed and weighed. The right kidney was frozen in liquid nitrogen and lyophilized for renal fluid content determination. The left kidney was fixed in alcoholic Bouin's reagent and embedded in paraffin blocks for cyst score determination. Four-micron sections were cut and stained with periodic acid–Schiff. A blinded individual performed the image analysis using Northern Eclipse (version 5.0) software (Empix Imaging, Inc, Toronto, Ontario, Canada). Cyst area was determined from five randomly selected fields (three fields in the cortex, two fields in the medulla), each representing 4.46 mm^2 of the kidney section. To give an estimate of cyst volume, cyst score was calculated by multiplying the percentage of cyst area by kidney weight and standardized for body weight, as previously described.[26] Creatinine and urea nitrogen concentrations in the serum and urine were measured using Sigma kits no. 640 and 555, respectively (Sigma Diagnostics, St Louis, MO).

Statistics

Data from affected animals were analyzed by two-way (gender × creatine supplementation) analysis of variance. If interactions were present, simple-effect differences were

determined using Tukey's post hoc test. Differences and interactions were considered significant at P less than 0.05.

RESULTS

Supplementing diets with creatine for 6 weeks resulted in greater disease progression and worsened renal function in Han:SPRD-*cy* rats with kidney disease. As cystic renal disease progresses, renal size and fluid content increase.[22,23,27] Kidney weights relative to body weights among creatine-supplemented males and females were 10% greater than in males and females fed the control diet (2.87 ± 0.08 versus 2.61 ± 0.09 g/100 g of body weight; $P = 0.037$). There is a marked sex dimorphism in the expression of the disease in this animal model that has been well-characterized in previous studies.[22,23] Therefore, individual group means and the overall P for the effect of creatine supplementation are listed in Tables 1 and 2, with the significant sex effects noted in the table legends. Kidney weights relative to liver weights were 13% greater in supplemented compared with nonsupplemented animals (0.78 ± 0.03 versus 0.69 ± 0.28 g/100 g of liver weight; $P = 0.023$). Significantly, the liver does not become cystic in these animals at this age, and creatine supplementation had no effect on the liver weights of either males or females. Body weights were similar in the control and supplemented groups (Table 1). In addition, food intakes were not different in the supplemented and control animals (data not shown).

Congruent with increased cyst fluid accumulation during disease progression, kidney water content was 2.1% greater in supplemented animals compared with animals fed the control diet (89.22 ± 0.41 versus 87.38 ± 0.48 g/100 g of tissue; $P = 0.006$), and cyst scores were 23% higher in creatine-supplemented animals (0.49 ± 0.02 versus 0.40 ± 0.03; $P = 0.017$; Table 1).

Markers of renal function also were affected by creatine supplementation. Serum urea concentration was 16% greater in supplemented compared with nonsupplemented animals (23.96 ± 0.92 versus 20.65 ± 1.06 mmol/L, respectively; $P = 0.023$; Table 2). For serum creatinine, concentrations were greater in supplemented animals, but the differences were statistically significant (33% greater) only in males. In supplemented animals, creatinine clearances were 23% less than in animals fed the control diet (0.125 ± 0.010 versus 0.162 ± 0.011 mL/min/100 g of body weight, respectively; $P = 0.0159$; Table 2). Urine concentrations of urea and creatinine at the

Table 1. Effect of Creatine Supplementation on Kidneys and Livers in Han:SPRD-*cy* Rats With Cystic Kidneys

	Male		Female		Creatine Effects
	Control Diet (n = 9)	Creatine-Supplemented Diet (n = 14)	Control Diet (n = 12)	Creatine-Supplemented Diet (n = 12)	($P < 0.05$)
Body weight (g)*	344.7 ± 5.7	343.6 ± 4.6	237.5 ± 5.0	245.4 ± 5.0	0.5064
Total kidney weight (g)*	10.73 ± 0.55	11.65 ± 0.44	5.02 ± 0.47	5.85 ± 0.48	0.0806
Relative kidney weight (g/100 g of body weight)*	3.11 ± 0.14	3.37 ± 0.11	2.11 ± 0.12	2.38 ± 0.12	0.0365
Total liver weight (g)*	13.83 ± 0.48	12.73 ± 0.38	8.71 ± 0.42	9.23 ± 0.41	0.4940
Relative liver weight (g/100 g of body weight)	4.00 ± 0.11	3.70 ± 0.09	3.66 ± 0.09	3.76 ± 0.09	0.2779
Relative kidney weight* (g/g of liver weight)	0.79 ± 0.04	0.92 ± 0.03	0.58 ± 0.04	0.63 ± 0.04	0.0233
Kidney water (g)*†	4.55 ± 0.23	4.84 ± 0.18	2.12 ± 0.19	2.61 ± 0.19	0.0566
Kidney water*† (g/100 g of tissue)	88.35 ± 0.74	89.68 ± 0.56	86.40 ± 0.61	88.76 ± 0.61	0.0057
Cyst area (%)*‡	35.57 ± 1.63	38.43 ± 1.16	20.30 ± 1.14	25.66 ± 1.83	0.0074
Cyst score*§	0.58 ± 0.04	0.67 ± 0.03	0.21 ± 0.04	0.30 ± 0.04	0.0167

NOTE. Data expressed as mean ± SEM.
*Significant effect of sex ($P < 0.05$).
†Right kidney only.
‡Cyst area is the percentage of the kidney section area occupied by cyst.
§Cyst score is the percentage of cyst area times left kidney weight (in grams) divided by body weight (in grams).

Table 2. Effect of Creatine Supplementation on Markers of Renal Function in Han:SPRD-*cy* Rats With Cystic Kidneys

	Male		Female		Creatine Effects ($P < 0.05$)
	Control Diet (n = 9)	Creatine-Supplemented Diet (n = 14)	Control Diet (n = 12)	Creatine-Supplemented Diet (n = 12)	
Serum urea (mg/dL)*	70.41 ± 4.61	82.69 ± 3.49	45.26 ± 3.77	51.52 ± 3.77	0.0230
Serum creatinine (mg/dL)	0.97 ± 0.06	1.28 ± 0.05†	0.71 ± 0.05	0.79 ± 0.05	Interaction‡
Creatinine clearance (mL/min/100 g body wt)	0.162 ± 0.016	0.128 ± 0.013	0.163 ± 0.014	0.121 ± 0.015	0.0159

NOTE. Data expressed as mean ± SEM.
*Significant effect of sex ($P < 0.05$).
†Significantly different from males fed the control diet ($P < 0.05$).
‡Interaction between creatine and sex effects ($P = 0.0415$).

end of the study were not significantly affected by supplementation (data not shown).

DISCUSSION

As the popularity of creatine supplements continues to increase in adult and pediatric populations, the importance of elucidating the potential health effects is paramount. Although controversy exists regarding detrimental effects on the kidney, there is a paucity of data on long-term effects and effects in individuals with already compromised renal function and those at risk for renal disease. Studies that indicate creatine supplements do not affect kidney function, as well as those suggesting detrimental effects, are limited by their study design and the number of participants. One short-term study indicated no apparent effects of creatine supplementation on kidney function after a brief exposure to this compound (5 days), although it is not known whether early undetected lesions may be present.[20] In a slightly longer study of women, serum urea concentrations were elevated after 8 weeks of supplementation and returned to baseline concentrations 4 weeks after supplementation ceased,[7] suggesting that creatine supplementation could influence renal function. In a longer retrospective study of a small number of elite athletes (n = 9) who used creatine supplements for an average of 2.7 years (range, 0.8 to 5 years), no effect on renal function was observed.[18] Although this study appears to suggest that renal function is preserved with creatine supplementation, this study is limited by the potential for individuals lacking adverse reactions to be the sole participants, the very low statistical power of this study, and the still relatively short length of the study.[19,21]

Two case reports have alluded to a detrimental effect of creatine supplementation in adult patients. In one case, a patient with relapsing nephrotic syndrome presented with deteriorating renal function that coincided with creatine intake,[15] and although renal function improved with cessation of creatine intake, no renal histological evaluation was obtained to support a causative role for creatine. In the other report, an otherwise apparently healthy patient presented with interstitial nephritis after oral creatine supplementation (20 g/d for 4 weeks). Impaired renal function and histological injury were apparent in this patient at presentation. However, after stopping creatine supplementation, renal function returned to normal.[17] Thus, although the widespread use and apparent lack of negative effects[2-6] may suggest that creatine supplementation has no short-term effects on the kidney, information regarding the long-term effects and potential interaction with renal disease is lacking.

In the present study, creatine supplementation resulted in increased disease progression and worsened renal function in the Han:SPRD-*cy* rat model of kidney disease. The dosage administered is similar on a body-weight basis to amounts routinely consumed by athletes in efforts to enhance athletic performance. Although results from animal models may not be directly extrapolated to humans, the shorter life span of the rat allows the possibility of examining effects in a relatively short time. In addition, if creatine supplementation has beneficial or detrimental effects,

using an animal model of renal disease would be expected to be more sensitive to alterations in the kidney. The level of creatine supplementation used in this study was slightly less than the level used in another rat study (3.3 g/kg of diet for 10 days), which showed enhanced running performance.[28]

The mechanism(s) by which creatine supplementation mediates its detrimental effects in this model of renal disease remains to be elucidated. Creatine may act as an osmolyte in cyst fluid and increase fluid secretion into renal cysts, thus exacerbating the disease process. Water retention occurs with creatine supplementation, and it has been suggested that one way in which creatine increases muscle volume is by increasing water retention in this tissue.[6] It also has been speculated that because creatine supplementation downregulates endogenous creatine synthesis, the potential accumulation of one of its precursors (arginine) may lead to hyperornithinemia and the subsequent accumulation of polyamines.[14,29] Elevated concentrations of these known growth promotors also may exacerbate the progression of polycystic kidney disease because one component of polycystic kidney disease is hyperproliferation of tubular epithelial cells.[30]

Glutamine is frequently combined with creatine in commercially manufactured sports nutrition products for its purported ability to improve creatine absorption. The greater levels of serum urea, however, cannot be explained by the greater levels of nitrogen in the supplemented diet. The addition of 0.4 g of creatine and 0.08 g of glutamine per kilogram of diet is negligible compared with the 36 g of glutamate included in the 174 g of protein per kilogram in the American Institute of Nutrition-93G (control) diet[25] and would not be sufficient to alter serum urea concentration. If renal disease were that sensitive to dietary nitrogen levels, the high-protein diets often consumed by athletes would be of concern. Higher protein diets have been found to increase disease progression and impair renal function in animal models of polycystic kidney disease.[26,31,32] In this regard, the total lack of knowledge of the potential additive or interactive effect of creatine supplementation with a high-protein diet or other ergogenic aids consumed by individuals is of concern.

Manifestations of polycystic kidney disease in humans do not present until the third to fifth decades of life.[27] Thus, if kidney function in these patients is adversely affected by creatine supplementation, individuals ingesting this compound unknowingly may be accelerating disease progression. Aside from being a specific model for polycystic kidney disease, the interstitial pathological characteristics and progressive nature of the disease present in the Han:SPRD-*cy* rat also makes research with this animal applicable to chronic interstitial nephritis, which has a role in 25% of all cases leading to renal failure.[33]

It is possible that creatine may not be detrimental to the healthy kidney, that damage to the kidney by creatine supplementation is clinically irrelevant to the healthy kidney, or that a lower dose that still has ergogenic effects may be benign to the kidney. However, even if creatine causes insignificant or no renal damage to a healthy kidney, it is not known whether it could potentially exacerbate renal deterioration if renal disease develops later in life. Clearly, long-term research using subjects with and at risk for renal disease is needed to assess the safety of regular oral intake of this very popular ergogenic aid.

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Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to

Creatine monohydrate for use in foods for particular nutritional uses

Question number EFSA-Q-2003-125

adopted on 17 February 2004

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to provide a scientific opinion, based on its consideration of the safety and bioavailability of the nutrient source, creatine monohydrate, when used in the manufacture of foods for particular nutritional uses.

Creatine occurs in the body, with higher concentrations in muscles. It can be obtained from the diet, predominantly meat and fish, and can be synthesized endogenously in the pancreas, kidneys and the liver from the amino acids glycine, arginine and methionine at the rate of 1-2 g/day.

A previous opinion from Scientific Committee on Food (SCF) expressed in 2000 considered the existing evidence as insufficient to provide reassurance about the safety of creatine supplementation involving high loading doses. It was indicated that little information exists on long-term safety of creatine, and that adequate quality control and adequate specifications for food grade materials should be developed.

A high purity (minimum 99.95%) source of creatine monohydrate is considered here. It is produced under conditions that prevent microbiological and heavy metals contamination, and acceptable limits for the impurities creatinine, dicyandiamide and dihydro-1,3,5-triazine are obtained.

The safety and bioavailability of the requested source of creatine, creatine monohydrate in foods for particular nutritional uses, is not a matter of concern provided that there is adequate control of the purity of this source of creatine with respect to dicyandiamide and dihydro-1,3,5-triazine derivatives. The Panel endorses the previous opinion of the SCF that high loading doses of creatine should be avoided. Provided high purity creatine monohydrate is used in foods for particular nutritional uses, the Panel considers that the consumption of doses of up to 3g/day of supplemental creatine, similar to the daily turnover rate of creatine, is unlikely to pose any risk.

KEY WORDS

Creatine monohydrate, Foods for particular nutritional uses (FPNU), CAS Registry Number 6020-87-7

BACKGROUND

Creatine (N-(aminoiminomethyl)-N-methyl glycine) is an endogenous substance, with the highest concentrations in the skeletal muscle (approximately 95% of the total creatine pool) and in the heart muscle. It occurs in foods such as meat, fish and other animal products. A typical diet supplies 1-2 grams of creatine daily. In the absence of dietary creatine it may also be formed endogenously by liver, kidney and pancreas from the amino acids glycine, arginine and methionine at the rate of 1-2 g/day (see references in SCF 2000a).

A report on "the composition and specification of food intended to meet the expenditure of intense muscular effort, especially for sportsmen" was adopted by the SCF in 2000 (SCF 2000b). It included creatine as a possible component of this particular food but the report addressed mainly efficacy and not safety issues.

Subsequently, an opinion of the SCF on safety aspects of creatine supplementation was expressed in 2000 (SCF 2000a). The SCF considered that supplementation with 2-3g/day, which are similar to the daily turnover rate of 2g/day, are unlikely to pose any risk.

The nutritional substances that may be added as sources of certain categories of nutrients are subject to Commission Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses (EC 2001) or directives on specific categories of foods for particular nutritional uses.

The SCF was asked in November 2001 to consider the safety of a number of substances as sources of nutrients for foods for particular nutritional uses (FPNUs). Due to deficiencies in the original dossiers submitted, the evaluations could not be completed under the SCF mandate and continuation of this work now falls to the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. Creatine monohydrate (CM) as a source of creatine was one of the substances.

Evaluation of CM as a nutrient source for creatine in practice includes evaluation of the purity of the source and the bioavailability of creatine from the source. Apart from the impurities the safety of CM is considered to be similar to that of creatine, the safety of which has been evaluated by the SCF before (SCF 2000a).

TERMS OF REFERENCE

The Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of the nutrient source, creatine monohydrate, when used in the manufacture of foods for particular nutritional uses.

ASSESMENT

Composition, specifications and properties.

CM has CAS-number 6020-87-7; its formula is $C_4H_9N_3O_2.H_2O$ and it has a molecular weight of 149.1. CM solubility in water is 1.3g/100g at 20 °C. CM contains 12.1 % water of crystallization. According to the petitioner (IDACE 2003) it is of high purity (typically 99.99%, min. 99.95%). The product is said to comply with the following specifications: creatinine maximum 100 mg/kg, dicyandiamide maximum 50 mg/kg and dihydro-1,3,5-triazine not detectable (lower than the detection limit of 4.5 mg/kg). It is produced under conditions, which prevent microbiological and heavy metals (maximum10 mg/kg) contamination (IDACE 2003). Maximum levels of each of Hg,

Cd, Pb or As are 1 mg/kg. The shelf life of CM is minimum 36 months from the date of manufacture, in an unopened container at room temperature.

Manufacturing process

The manufacturing process of this product (chemical synthesis from cyanamide and sarcosine) is described by the petitioner (US patent 5,719,319).

The petitioner indicates that the reaction conditions as well as the treatment of the crude CM are crucial for the quality of the product. Inferior starting materials or insufficient amount of water during "recrystallization" result in increased amounts of impurities, such as dicyandiamide, creatinine and dihydro-1,3,5-triazine derivatives.

Proposed use

The proposed use for CM as a source of creatine is in food for particular nutritional uses intended for all age groups. The petitioner provided no information on intended levels of use.

Exposure

The petitioner gives no information on exposure to CM. Typical doses of pure creatine monohydrate used in scientific studies range between 2-25 grams per person per day for an average adult and the dosing regimens suggested by the manufacturers of creatine are 20 g/day for 3 to 7 days and then 2-5 g/day as a maintenance dose (see SCF 2000a).

Existing authorizations

It is reported (IDACE, 2003) that CM has been used as a supplement, for example in Germany (10g/day) and the USA. In the USA CM is being used as a dietary supplement under the conditions of the Dietary Supplement Health and Evaluation Act (DSHEA) and it can be purchased over the counter in health food stores and pharmacies (IDACE 2003).

Biological and toxicological data

Bioavailability and interactions

According to the petitioner, ingestion of 5 g of CM in humans produces an increase in plasma creatine over 500 µM (0.75 g/l) one hour after ingestion, which indicates that CM is bioavailable.

Toxicological information:

Only a limited summary of toxicological information was supplied by the petitioner reporting that the acute toxicity of CM is low (LD_{50} in the rat is higher than 2 g/kg), and that it is not mutagenic in the Ames test. CM has been tested in a 28-day rat study in which no treatment related adverse effects were reported after dose levels up to 2 g/kg bw/day.

A recent study (Kreider *et al.* 2003) examined the effects of long-term creatine supplementation on several serum, whole blood, and urinary markers of clinical health status in athletes (range 18-23 years), over a 21-month period. Subjects were given 15.75 g/day of CM for 5 days and an average of 5 g/day thereafter in 5-10 g/day doses. A comprehensive quantitative clinical chemistry set of parameters was determined on serum and whole blood samples (metabolic markers, muscle and liver enzymes, electrolytes, lipid profiles, haematological markers, and lymphocytes). In addition,

urine samples were used to assess clinical status and renal function. Baseline and the subjects' final blood and urine samples were analyzed. At the end of the study, subjects were categorized into groups that did not take creatine (n = 44) and subjects who took creatine for 0-6 months (mean 4.4 +/- 1.8 months, n = 12), 7-12 months (mean 9.3 +/- 2.0 months, n = 25), and 12-21 months (mean 19.3 +/- 2.4 months, n = 17). There were no significant differences among the groups in the 54-item panel of quantitative blood and urine markers assessed. The results indicate that creatine supplementation for up to 21 months does not appear to adversely affect markers of health status in athletes undergoing intense training in comparison to athletes who do not take creatine (Kreider *et al.* 2003).

A tolerable daily intake (TDI) of 1 mg/kg bw/day has been established for dicyandiamide used as a monomer for food packaging material (SCF 1995).

Discussion

No specific assessment of exposure of CM was considered necessary provided that the intake of creatine is within the amounts judged unlikely to pose any risk (2-3 g/day).

The bioavailability of CM is considered to be the same as that of creatine.

Daily intake of 3 g of CM containing the highest level of impurities specified would lead to an intake of less than 150 µg of dicyandiamide/day. Considering a human body weight of 60 kg, such an intake would be less than 2.5 µg/kg bw/day of dicyandiamide, i.e. 0.25% of the TDI of 1 mg/kg bw/day established for this substance for use as a monomer for food packaging material (SCF 1995). Dihydro-1,3,5-triazine derivatives were reported to be not detectable in the CM preparation under consideration (detection limit of 4.5 mg/kg).

The safety of CM is therefore considered to be similar to that of creatine, which has been considered earlier by the SCF (SCF 2000a) who concluded the following:

"Although many efficacy trials have studied the effects of creatine, large-scale, well-controlled studies are lacking. Available results observed in highly trained athletes cannot necessarily be extrapolated to the general public. Little information exists on the short-term or long-term safety of creatine and adequate quality control of the commercially marketed creatine is lacking. Adequate specifications for food grade materials should be developed.

Although no important adverse effects have been reported in the efficacy trials, such evidence is insufficient to provide reassurance about the safety of creatine supplementation involving high loading doses: there are doubts about safety in relation to kidney function; studies on tissues in which creatine is known to concentrate are lacking; effects on endogenous creatine synthesis upon cessation of supplementation are also not well studied. For these reasons the Committee considers that high loading doses should be avoided. Consumption of lower doses of around 3g/day are similar to the daily turnover rate of 1-2g/day and are unlikely to pose any risk.

Future studies should evaluate short- and long-term effects of oral creatine on renal and hepatic systems as well as those organs where creatine plays a metabolic role. Such studies should include people who are not highly trained.

CM has been tested in a 28-day rat study. No treatment related adverse effects were reported after dose levels up to 2 g/kg bw/day.

The results of a recent human study indicate that CM intake (~ 5 g/day for up to 21 months) appears to be safe for athletes engaged in intense training and competition. However, this does not give reassurance about potential long-term effects of high doses of CM in people who are not highly trained or belong to other population subgroups.

CONCLUSION

The safety and bioavailability of the requested source of creatine, creatine monohydrate, in foods for particular nutritional uses, is not a matter of concern provided that there is adequate control of the purity of this source of creatine with respect to dicyandiamide and dihydro-1,3,5-triazine derivatives.

The Panel endorses the previous opinion of the SCF that high loading doses of creatine should be avoided. Provided high purity creatine monohydrate is used in foods for particular nutritional uses, the Panel concurs with the previous opinion of the SCF that the consumption of doses of up to 3g/day of supplemental creatine, similar to the daily turnover rate of creatine, is unlikely to pose any risk.

DOCUMENTATION PROVIDED TO EFSA

Letter from the European Commission to the Chairman of the Scientific Committee on Food on commission request for a specific opinion on the evaluation of a number of substances added for specific nutritional uses in foods for particular nutritional uses. SANCO D4/AN/dlc-D(2003)440384

Submission by IDACE (Paris, France) in reference to Commission Directive 2001/15/EC on substances that may be added for specific nutritional uses on foods for particular nutritional uses. Title: Creatine monohydrate dossier. Paris 2003

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* Declaration of interest see minutes of the 5th meeting of the AFC Panel held on 16 and 17 February 2004. These can be found at http://www.efsa.eu.int/science/afc/afc_meetings/catindex_en.html

European Food Safety Authority

The EFSA Journal (2008) 689, 1-79

Nitrate in vegetables

Scientific Opinion of the Panel on Contaminants in the Food chain[1]

(Question N° EFSA-Q-2006-071)

Adopted on 10 April 2008

PANEL MEMBERS

Jan Alexander, Diane Benford, Andrew Cockburn, Jean-Pierre Cravedi, Eugenia Dogliotti, Alessandro Di Domenico, María Luisa Fernández-Cruz, Johanna Fink-Gremmels, Peter Fürst, Corrado Galli, Philippe Grandjean, Jadwiga Gzyl, Gerhard Heinemeyer, Niklas Johansson, Antonio Mutti, Josef Schlatter, Rolaf van Leeuwen, Carlos Van Peteghem, Philippe Verger.

SUMMARY

Nitrate is a naturally occurring compound that is part of the nitrogen cycle, as well as an approved food additive. It plays an important role in the nutrition and function of plants. Nitrate is an important component of vegetables due to its potential for accumulation; this can be affected by a number of biotic and abiotic factors. Higher levels of nitrate tend to be found in leaves whereas lower levels occur in seeds or tubers. Thus leaf crops such as lettuce and spinach generally have higher nitrate concentrations. Human exposure to nitrate is mainly exogenous through the consumption of vegetables, and to a lesser extent water and other foods. Nitrate is also formed endogenously. In contrast exposure to its metabolite nitrite is mainly from endogenous nitrate conversion.

Nitrate *per se is* relatively non-toxic, but its metabolites and reaction products e.g., nitrite, nitric oxide and N-nitroso compounds, have raised concern because of implications for adverse health effects such as methaemoglobinaemia and carcinogenesis. On the other hand recent research

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[1] For citation purposes: Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables, *The EFSA Journal* (2008) Journal number, 689, 1-79.

indicates that nitrite participates in host defence having antimicrobial activity, and other nitrate metabolites e.g. nitric oxide, have important physiological roles such as vasoregulation. Despite being a major source of nitrate, increased consumption of vegetables is widely recommended because of their generally agreed beneficial effects for health.

In order to provide a strategy to manage any risks to human health from dietary nitrate exposure resulting from vegetable consumption an updated risk assessment was requested from the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) by the European Commission. The opinion was to take into account the amounts of nitrate found in vegetables as consumed and any relevant considerations on the possible balance between risks and benefits.

As a response to a call for data on nitrate levels in vegetables, EFSA received 41,969 analytical results from 20 Member States and Norway. There was a large variation in median concentrations of nitrate in different vegetables from a low of 1 mg/kg (peas and Brussels sprouts) to a high of 4,800 mg/kg (rucola). Less than 5% of all samples were reported as being below the limit of detection (LOD) for nitrate. A reasonable approximation of European vegetable consumption was estimated from the GEMS/Food Consumption Cluster Diets database and consumption data submitted by EU Member States. In consequence, a base case of vegetable and fruit intake of 400 g/person/day, as recommended by the World Health Organization (WHO), was used, but considered to be all in the form of vegetables. In addition, from the data collected, different scenarios combining a range of consumption patterns with concentration of nitrates in the relevant food category were estimated. The scenarios demonstrated that the critical driver for a high dietary exposure to nitrate is not the absolute amount of vegetables consumed but the type of vegetable (e.g. leafy vegetables) and the concentration of nitrate related to the conditions of production.

An Acceptable Daily Intake (ADI) for nitrate of 3.7 mg/kg b.w./day, equivalent to 222 mg nitrate per day for a 60 kg adult was established by the former Scientific Committee on Food (SCF) and was reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002. The CONTAM Panel noted that no new data were identified that would require a revision of the ADI.

To assess any potential health impacts from the different vegetable intake scenarios the CONTAM Panel compared the nitrate exposure estimates with the ADI for nitrate of 222 mg/day for a 60 kg human. Additionally, to place these findings in context, exposures from other nitrate sources such as drinking water and cured meat, at an average of 35-44 mg/person per day, were also taken into account. As a conservative base case, a person eating 400 g of mixed vegetables at typical median nitrate concentration levels would on average receive a dietary exposure to nitrate of 157 mg/day. This is within the ADI even when the exposure to nitrate from other dietary sources is considered. Considering that for most people, fruit, which has low nitrate levels in the order of 10 mg/kg, comprises up to one half of the total recommended daily intake of 400 g of vegetables and fruit, actual nitrate intakes would be reduced to between 81-106 mg/day for the majority of the EU population. Further mitigation of nitrate intake may result from processing e.g. washing, peeling and/or cooking.

A small part of the population (2.5%) in some Member States eats only leafy vegetables and in high amounts, which can lead to the ADI being exceeded. Should the vegetables be produced under unfavourable growing conditions the ADI could be exceeded by approximately two fold. The Panel also noted that consumption of more than 47 g of rucola at the median nitrate concentration would lead to an excursion above the ADI without taking into account any other source of nitrate exposure.

Epidemiological studies do not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk. Evidence that high intake of nitrite might be associated with increased cancer risk is equivocal.

The Panel compared the risk and benefits of exposure to nitrate from vegetables. Overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail. The Panel recognised that there are occasional circumstances e.g. unfavourable local/home production conditions for vegetables which constitute a large part of the diet, or individuals with a diet high in vegetables such as rucola which need to be assessed on a case by case basis.

Key Words

Nitrate, nitrite, vegetables, ADI, risk assessment, risk benefit analysis, human health

TABLE OF CONTENT

BACKGROUND AS PROVIDED BY REQUESTOR

The Scientific Committee for Food (SCF) set an Acceptable Daily Intake (ADI) for nitrate of 0-3.7 mg/kg body weight in 1995. It is possible that this ADI might be exceeded in some cases, for example based upon levels found in vegetables and quantities that are consumed.

The European Commission set maximum levels for nitrate in lettuce and spinach (Regulation (EC) 1881/2006[2]. These levels are regularly reviewed using monitoring data from the Member States. In some cases, despite developments in good agricultural practice, the maximum levels can be exceeded, particularly for spinach. In addition, the implications for food safety from nitrate in other vegetables are unclear. For example, rucola (often called rocket in English) has been found to regularly contain high levels of nitrate, whereas potatoes often contain lower levels of nitrate, but are eaten more frequently and so dietary intake can be significant. Thus the amounts of nitrate in vegetables might sometimes lead to the ADI being exceeded, although it is unclear whether cooking could significantly lower the nitrate levels in the products as eaten.

The significance of the levels of nitrate found in vegetables (e.g. lettuce, spinach, rucola, potatoes and others) should be assessed, taking into account the effects of preparation, such as usual cooking procedures, on the levels of nitrate or relevant metabolites that may be present in the vegetables as normally consumed.

To provide an up-to-date scientific basis for the longer-term strategy for managing the risk from nitrate in vegetables, a scientific risk assessment is needed from the European Food Safety Authority taking into account new information generated since the opinion of the SCF in 1995. The assessment should take into account any relevant considerations on risks and benefits, for example to weigh the possible negative impact of nitrate versus the possible positive effects of eating vegetables, such as antioxidant activities or other properties that might in some way counteract or provide a balance to the risks from nitrate and the resulting nitroso-compounds.

TERMS OF REFERENCE AS PROVIDED BY REQUESTOR

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to assess the risks to consumers from nitrate in vegetables. The assessment should take into account the amounts of nitrate found in vegetables as consumed and any relevant considerations on the possible balance between risks and beneficial health effects.

2 OJ L 364, 20.12.2006, p. 5.

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ASSESSMENT

1. Introduction

Almost 80 % of the earth's atmosphere consists of nitrogen as the most abundant chemical element. Nitrogen is also a key component of essential biomolecules such as amino acids, vitamins, hormones, enzymes, and nucleotides. In living tissues, nitrogen is ranked quantitatively as the fourth most common element behind carbon, oxygen and hydrogen and is an integral part of the nitrogen cycle, which continuously interchanges nitrogen between organisms and the environment.

Nitrate is a naturally occurring compound and is an important component of vegetables because of its potential to accumulate. It is formed naturally in living and decaying plants and animals, including humans (Mensinga *et al.,* 2003; Lundberg *et al.,* 2004 and 2008; Camargo and Alonso, 2006). Nitrate is also used in agriculture as a fertilizer to replace the traditional use of livestock manure and in food processing as an approved food additive. Nitrate *per se* is relatively non-toxic, but its metabolites, nitrite, nitric oxide and N-nitroso compounds, make nitrate of regulatory importance because of their potentially adverse health implications. On the other hand recent research shows that its conversion to nitrite plays an important antimicrobial role in the stomach (McKnight, *et al.*, 1999), and other nitrate metabolites also have important physiological/pharmacological roles (Lundberg *et al.*, 2004, 2006 and 2008; Bryan *et al.*, 2005).

The first international evaluation of the risks associated with the ingestion of nitrate and nitrite was conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1961 (FAO/WHO, 1962). The Scientific Committee for Food reviewed the toxicological effects of nitrate and nitrite and established an Acceptable Daily Intake (ADI) of 0-3.7 mg/kg b.w. for nitrate in 1990 (EC, 1992), retained the ADI in 1995 and derived an ADI of 0-0.06 mg/kg for nitrite (EC, 1997). The JECFA completed its most recent review in 2002 and reconfirmed an ADI of 0-3.7 mg/kg b.w. for nitrate and set an ADI of 0-0.07 mg/kg b.w. for nitrite (FAO/WHO, 2003a,b).

Nitrate predominately enters the human body exogenously from vegetables, water, and other foods, but is also formed to a limited extent endogenously (Lundberg *et al.,* 2004 and 2008).

Some vegetables, particularly leafy vegetables, have been shown to have relatively high levels of nitrate, but implications for food safety are unclear. In order to provide a scientific basis for a longer-term strategy to manage any risks from dietary nitrate an updated risk assessment was considered necessary by the European Commission.

Nitrate and plants

Nitrogen is the main growth-limiting factor in most field crops and the major source in plants is mineralised nitrogen, as nitrate and ammonium. Farmers may therefore use manure and nitrogen-based fertilizers to boost crop yields. A range of leafy vegetables can accumulate high levels of nitrate. The concentrations depend on a range of factors including season, light, temperature, growing conditions, fertilizer use, and storage of the crop (Dich *et al.,* 1996). In Europe there is a tendency for the concentrations to be higher in more northerly latitudes and during the winter, owing to the lower light intensity and fewer daylight hours.

Nitrate and water

If Good Agricultural Practice (GAP) is not followed, the application of nitrogen fertiliser and/or manures can result in increased concentrations of inorganic nitrogenous compounds in ground and surface waters. International efforts have been put in place to reduce and limit the occurrence of nitrate in water. In the European Union, the first harmonised standards concerning the quality of surface water intended to be used as drinking water were laid down in 1975 in the Council Directive 75/440/EEC[3]. The current maximum level of 50 mg nitrate/L in drinking water is laid down in Council Directive 98/83/EC[4].

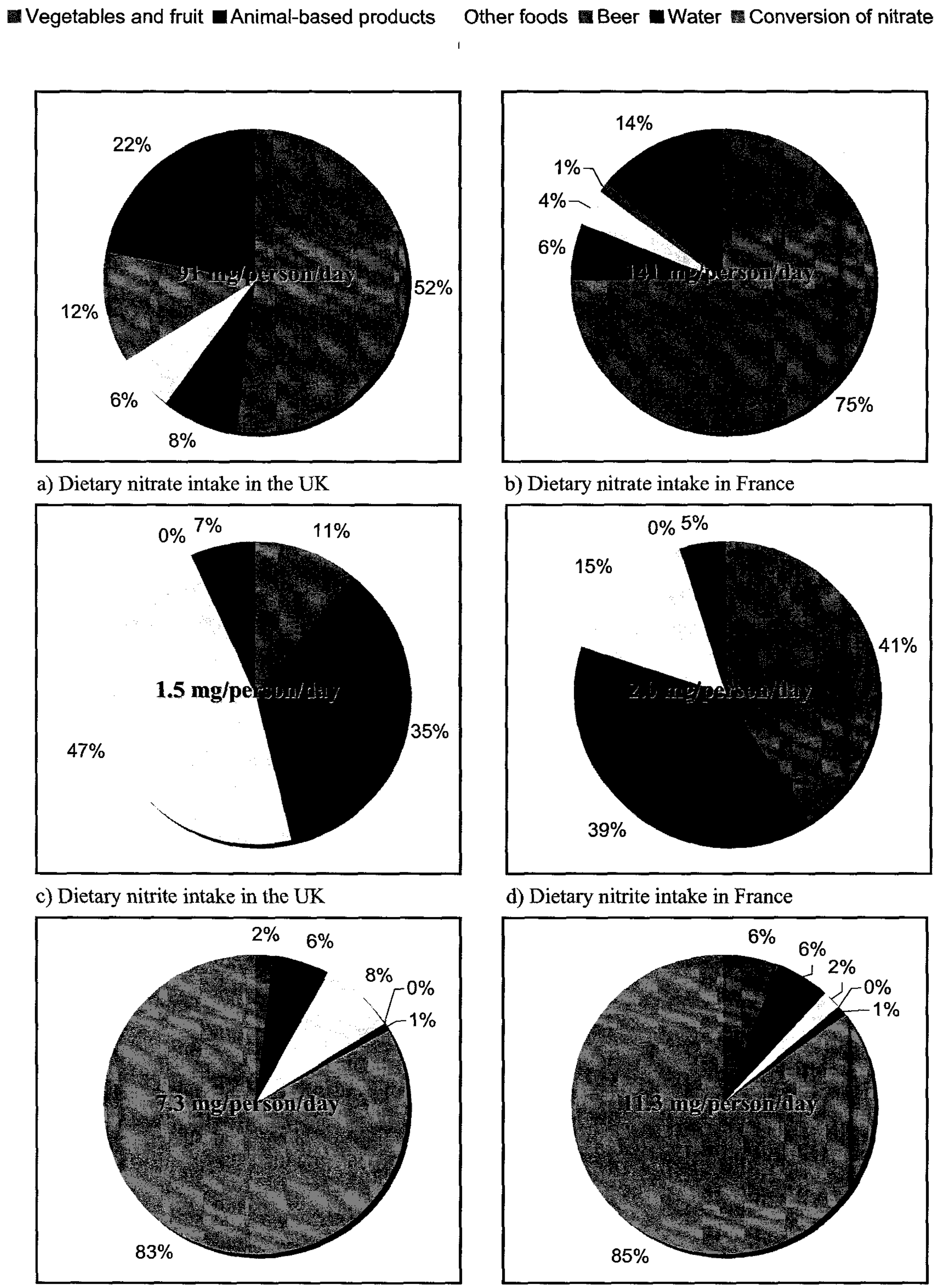
Sources of nitrate and nitrite exposure

While human exposure to nitrate is mainly exogenous, exposure to nitrite is mainly endogenous via nitrate metabolism. Some nitrite is consumed as a consequence of its use as a food preservative and to a lesser extent from its presence in vegetables. Figure 1 illustrates the estimated total daily dietary exposure for both nitrate and nitrite, expressed as a percentage of the total diet, for the UK as an example of a Northern European country (MAFF, 1998a,b) and France as an example of a Central/Southern European country (modified after Causeret, 1984). For both the UK and France the most important sources of dietary intake of nitrate are vegetables and fruit contributing 50 to 75% to the overall dietary intake (see Figures 1a and 1b). Also for both countries the largest source of nitrite is endogenous conversion from nitrate (see Figures 1e and 1f). A conservative factor of 7% for the ingested nitrate to nitrite conversion was used for the calculations (see chapter 8.1).

Although fruit and vegetables contribute 11-41% of exogenous nitrite dietary intake (see Figures 1c and 1d), this amount is overshadowed by the endogenous reduction of secreted salivary nitrate to nitrite. Thus the exposure assessment in this opinion will focus mainly on nitrate concentrations in vegetables and consider nitrite only when dealing with the total body burden of nitrate and its metabolites with regard to any implications for human health.

[3] OJ L 194, 25.7.1975, p. 26-31

[4] OJ L 330, 5.12.1998, p. 32-54 corrigendum OJ L 111, 20.4.2001, p. 31



a) Dietary nitrate intake in the UK

b) Dietary nitrate intake in France

c) Dietary nitrite intake in the UK

d) Dietary nitrite intake in France

e) Total nitrite exposure(including endogenous conversion from nitrate) in the UK

f) Total nitrite exposure (including endogenous conversion from nitrate) in France

Figure 1. Relative intake contribution for sources of nitrate and nitrite in the UK and France.

Nitrate in humans and laboratory animals

The toxicokinetics of nitrate are complex (see chapter 8.1). In humans, dietary nitrate is rapidly absorbed via the stomach and the proximal intestine into the plasma and at least 25% is transported into the saliva. The salivary nitrate concentration is approximately 10-fold that of plasma due to bio-concentration (McKnight *et al.,* 1999). On the surface of the tongue, commensal bacteria reduce approximately 20% of the secreted nitrate into nitrite (Lundberg *et al.,* 2004 and 2008) which is then swallowed along with the unconverted nitrate. Healthy adults have a salivary conversion of nitrate to nitrite of normally 5-7% of the total nitrate intake, whereas infants and patients with gastroenteritis who have a higher gastric pH can have a considerably greater conversion rate.

Nitrate is relatively non-toxic, the main toxicological endpoints in laboratory animals result from the formation of nitrite and its ability to react to form N-nitroso compounds. Several toxicological effects have been identified: methaemoglobin formation, hyperplasia of the zona glomerulosa of the adrenal cortex and gastric neoplasia.

Definition of vegetables

The focus of this opinion is on vegetables. The definition of the word vegetable is traditional rather than scientific and is somewhat arbitrary and subjective. All parts of herbaceous plants eaten as food by humans, whole or in part, are normally considered vegetables. Mushrooms, though belonging to the biological kingdom fungi, are also commonly considered vegetables. Tubers (like potato) are included in the definition of vegetables in some countries but not in others. Nuts, grains, herbs, spices and culinary fruits are normally not considered as vegetables. Botanically, fruits are reproductive organs, while vegetables are vegetative organs which sustain the plant. Nevertheless, several fruits, e.g. cucumbers, are also included in the term vegetables.

A formal definition of fruits and vegetables was attempted by the World Health Organisation for use in epidemiological studies (IARC, 2003). They are defined as "edible plant foods excluding cereal grains, nuts, seeds, tea leaves, coffee beans, cocoa beans, herbs and spices". Fruits are "edible parts of plants that contain the seeds and pulpy surrounding tissue; have a sweet or tart taste; generally consumed as breakfast beverages, breakfast and lunch side-dishes, snacks or desserts." Vegetables are "edible plant parts including stems and stalks, roots, tubers, bulbs, leaves, flowers, and fruits; usually include seaweed and sweet corn; may or may not include pulses or mushrooms; generally consumed raw or cooked with a main dish, in a mixed dish, as an appetiser, or in a salad".

For this opinion vegetables were taken to be leaves (e.g. lettuce), stems (e.g. asparagus), roots (e.g. carrots), flowers (e.g. broccoli), bulbs (e.g. garlic), seeds (e.g. peas and beans) and botanical fruits such as cucumbers, squash, pumpkins, and capsicums. Tubers are included in the general definition but also presented separately. Herbs are presented in a separate table but not included in the overall definition of vegetables.

Risk benefit analysis

Risk-benefit analysis of foods with regard to human health is a developing area and no common paradigm on the general principles or approaches for conducting a quantitative risk-benefit analysis for food and food ingredients has been established yet at either a European or international level. Within the EU recently a number of projects such as Qalibra[5], Beneris[6] and Brafo[7] have been initiated to progress the science, tools, methods and implications of risk-benefit analysis. At a recent EFSA Scientific Colloquium on Risk-benefit Analysis of Foods – Methods and Approaches (EFSA, 2007) it was concluded that risk-benefit analysis should be symmetrical and thus mimic the classical risk assessment and risk analysis paradigms. The same steps should be involved, namely health benefit identification, health benefit characterisation and exposure assessment in order to arrive at an overall benefit characterisation. The outcome of the respective risk characterisation and benefit characterisation would then drive an integrated risk-benefit analysis via risk-benefit assessment, risk-benefit management and risk-benefit communication.

2. Legislation on nitrate

Maximum levels for nitrate in vegetables were first set in the EU in 1997 by Commission Regulation (EC) No 194/97[8]. Since then, the Regulation has been amended several times. The current maximum levels are laid down in the Annex, Section 1 of Commission Regulation (EC) No 1881/2006[2] of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs[9] (Table 1) The Regulation on nitrate applies for the following five food commodities: fresh spinach, preserved, deep-frozen or frozen spinach, fresh lettuce (protected and open-grown lettuce), iceberg-type lettuce and processed cereal-based foods and baby foods for infants and young children. All maximum levels are expressed as mg nitrate/kg fresh weight.

Because of widely varying climatic conditions, production methods and eating habits in different parts of the European Union, maximum levels for fresh spinach and fresh lettuce are fixed depending on the season. The maximum levels for nitrate in those foodstuffs that are harvested between 1 October and 31 March are generally higher than the respective levels for samples harvested between 1 April and 30 September. Moreover, with respect to fresh lettuce the Regulation differentiates between lettuce grown under cover and lettuce grown in the open air with lower levels for the latter commodities.

[5] Available at URL: http://www.qalibra.eu/
[6] Available at URL: http://www.beneris.eu/
[7] Available at URL: http://europe.ilsi.org/activities/ecprojects/BRAFO/default.htm
[8] OJ L 31, 1.2.1997, p. 48
[9] OJ L 364, 20.12.2006, p. 5-24

Table 1. Maximum levels for nitrate as laid down in Commission Regulation (EC) No 1881/2006[2].

Foodstuff	Maximum level (mg nitrate/kg)	
Fresh spinach *(Spinacia oleracea)*	Harvested 1 October to 31 March Harvested 1 April to 30 September	3,000 2,500
Preserved, deep-frozen or frozen spinach		2,000
Fresh lettuce *(Lactuca sativa* L.) (protected and open-grown lettuce) excluding lettuce listed below	Harvested 1 October to 31 March: lettuce grown under cover lettuce grown in the open air Harvested 1 April to 30 September lettuce grown under cover lettuce grown in the open air	 4,500 4,000 3,500 2,500
Iceberg-type lettuce	Lettuce grown under cover Lettuce grown in the open air	2,500 2,000
Processed cereal-based foods and baby foods for infants and young children		200

By way of derogation several Member States are allowed for a transitional period (until 31 December 2008) to authorize the marketing of spinach or lettuce grown and intended for consumption in their territory with nitrate levels higher than the levels fixed in Regulation (EC) No 1881/2006[9]. However, lettuce and spinach producers in the Member States which have been given the aforementioned authorisation should progressively modify their farming methods in order to minimise the nitrate content by applying the good agricultural practices (GAP – see also chapter 4) recommended at the national level.

3. Sampling and methods of analysis

As sampling, sample preparation and analytical procedures play an important role for a reliable nitrate determination, general criteria were set in the EU in 1997 by Commission Regulation (EC) No 194/97[10]. These provisions were amended several times and are presently fixed in Commission Regulation (EC) No 1882/2006 of 19 December 2006[11] laying down methods of sampling and analysis for the official control of the levels of nitrate in certain foodstuffs. Besides definitions and general provisions this Regulation stipulates detailed requirements for methods of sampling for the different food commodities. No requirements exist for the number of samples that have to be analysed. Article 9 of Commission Regulation (EC) No 1881/2006[9] setting maximum levels for certain contaminants in foodstuffs only requests that Member States shall monitor nitrate levels in vegetables which may contain significant levels, in particular green leaf vegetables, and communicate the results to the Commission by 30 June each year. As a general obligation, according to Regulation (EC) No 882/2004[12] on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules,

[10] OJ L 31, 1.2.1997, p. 48-50
[11] OJ L 364, 20.12.2006, p. 25
[12] OJ L 165, 30.4.2004, p. 1-141 + corrigendum

Member States shall ensure that official controls are carried out regularly, on a risk basis and with appropriate frequency.

Commission Regulation (EC) No 1882/2006[9] also contains strict requirements with which the methods of analysis have to comply in order to ensure that control laboratories use procedures with comparable levels of performance. The Regulation follows the "criteria approach". This means that no prescribed fixed official methods have to be followed but laboratories can use each method of analysis, provided it can be demonstrated in a traceable manner that they strictly fulfil the analytical requirements laid down in the respective legislation. As a general requirement, methods for nitrate analysis used for food control purposes must comply with the provisions of items 1 and 2 of Annex III (characterisation of methods of analysis) to Regulation (EC) No 882/2004[12] of the European Parliament and of the Council of 29 April 2004[13] on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. While Regulation (EC) No 882/2004[12] contains the general provisions, the specific requirements for the official control of nitrate in vegetables are laid down in Commission Regulation (EC) No 1882/2006[9]. The latter sets performance criteria for recovery and precision. The "recommended" recovery values for the concentration values <500 mg/kg and ≥500 mg/kg are set as 60-120% and 90-110%, respectively. Moreover, annex D.3.2 of Commission Regulation (EC) No 1882/2006[9] states that the precision values have to be calculated at the concentration of interest from the Horwitz equation[14]. While the recommended value is the one derived from the Horwitz equation, the maximum permitted value is two times the value from the Horwitz equation.

Depending on type of foodstuff and concentration of interest a variety of analytical methodologies and principles are applicable. Some methods have already been standardized for the determination of nitrate and nitrite in various foodstuffs by the European Committee for Standardization (CEN). Within its technical committee CEN/TC 275 "Food analysis – horizontal methods" standards were elaborated for the determination of nitrate and nitrite in vegetables, vegetable products, including vegetable containing food for babies and infants as well as in meat and meat products. The respective methods are published in the standard series EN 12014, parts 1, 2, 3, 4, 5, and 7.

Finally, the Commission Regulation (EC) No 1882/2006[9] sets requirements for the reporting of results and the assessment of compliance of the lot or sub lots. For this, the analytical result corrected for recovery shall be used for checking compliance. The analytical result must be reported as $x \pm U$ whereby x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 %.

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[13] OJ L 191, 28.5.2004, p. 1

[14] The Horwitz equation is a generalised precision equation which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

4. Factors influencing the concentration of nitrate in plants

Nitrogen is essential to the nutrition and function of plants, so plants exert a close metabolic control on the concentration of nitrate and other nitrogen compounds. Nitrate is mainly to be found in cell vacuoles and is transported in the xylem. The xylem carries water and nutrients from the roots to the leaves, whereas the phloem carries the products of photosynthesis from the leaves to the growth points of the plant. This affects the distribution of nitrate between the leaves and storage organs such as seeds or tubers. This means that leaf crops such as cabbage, lettuce and spinach have fairly large nitrate concentrations whereas storage organs such as potato tubers, carrots, leeks, onions, seeds and pods of pea and bean plants have relatively small concentrations.

Another consequence of the transport system is that young leaves have lower nitrate concentration than older leaves. Such a relation was shown for cabbage with greatest nitrate concentrations in the outer leaves and much smaller nitrate concentration in the innermost leaves (Greenwood and Hunt, 1986).

Both environmental and agricultural factors can influence the nitrate concentrations in vegetables. The former include soil moisture, light intensity and temperature and the latter fertilizers, variety and crop protection strategies. The codes of GAP take these factors into consideration, region by region, in order to minimise nitrate levels in crops.

Soil

Nitrate moves from the bulk soil to the root surface mainly by convection rather than diffusion, so shortage of water will restrict nitrate uptake. Excess soil water dilutes the nitrate in the soil solution and can make the soil anoxic, thereby restricting crop growth and causing loss of nitrate by denitrification. Soil type and mineral content can affect nitrate accumulation.

Light intensity

The coupling of nitrogen assimilation and photosynthetic electron transport in leaves implies that light intensity is the key factor in determining nitrate concentrations in leaf crops. Month to month differences in light intensity caused as much as threefold variations in nitrate concentrations in lettuce grown in Western Europe (Van Eysinga, 1984). Winter-sown crops have generally higher nitrate concentration than summer crops in the same environment and Northern European crops have higher nitrate levels compared to Southern European crops (Weightman *et al.* 2006, AFFSA, 2007). These differences can be explained by both higher irradiance in summer which tends to reduce nitrate, and also to higher growth rates which coincide with periods of high irradiance and warmer temperatures (Kanaan and Economakis 1992). Current UK crop assurance protocols therefore suggest that growers should avoid sampling lettuce during dull weather conditions or during a particular time of the day (Anonymous 2002). The maximisation of light availability influences also the level of nitrate when crops are produced under glasshouse conditions (Premuzic *et al.*, 2002), this means e.g. shading of crops should be avoided.

Nitrogen fertilizer

Nitrogen fertilizer may contain nitrogen as nitrate, ammonium or urea and occasionally other forms. Once in the soil the other forms will mainly be converted to nitrate. Applying nitrogen fertilizer increases nitrate concentrations in the xylem but has virtually no effect on concentrations in the phloem. Leaf crops such as lettuce or cabbage therefore show an increased concentration of nitrate in response to nitrogen fertilizer, except in their very youngest leaves, while storage organs such as peas and beans that are fed by the phloem tend to show little effect. Nitrate concentrations in soil-growing storage roots that have very small nitrate concentrations will respond little to nitrogen.

Good agricultural practice for minimizing nitrate concentrations in vegetables

Schemes for GAP with regard to nitrate were developed to help farmers respond to the European nitrate regulations and the need to minimise nitrate concentrations in vegetables. They were produced by the Member States themselves, vegetable growers' organisations or commercial interests. Each GAP comprises an assembly of currently available knowledge, including recommendations based on experiments, and is intended to address environmental, agricultural, economic and social sustainability issues in on-farm production and the processing of produce beyond the farm gate.

A range of different GAP schemes operate within the Member States, each of which takes into account the particular climatic conditions in that Member State. All the schemes focus on abiotic factors shown to have a significant effect on nitrate concentrations in plants. First among these in almost all GAP schemes is adequate light intensity, particularly for vegetables grown under glass or plastic sheeting. GAP for nitrogen nutrition also aims to minimize 'untimely nitrate', that is, nitrate that is in the soil when it is not needed by the crop. The finding that nitrate concentrations in the outer leaves of lettuce are greater than those in inner leaves leads to another common element in GAP, the recommendation that growers should aim for large head weights to allow some trimming where appropriate. Other advice concerns *inter alia* analyses of the growing medium, choice of cultivars and the time between harvest and sale. The latter needs to be as short as possible to prevent water loss, which would be expected to increase the nitrate concentration on a weight for weight basis in the produce.

5. Occurrence of nitrate in vegetables

A call for detailed information on nitrate concentrations in individual vegetable samples was issued by the European Commission to EU Member States in November 2006. In total, EFSA received 41,969 analytical results from 20 Member States and Norway covering the period from 2000 to 2007. The sample distribution across countries is shown in Figure 2.

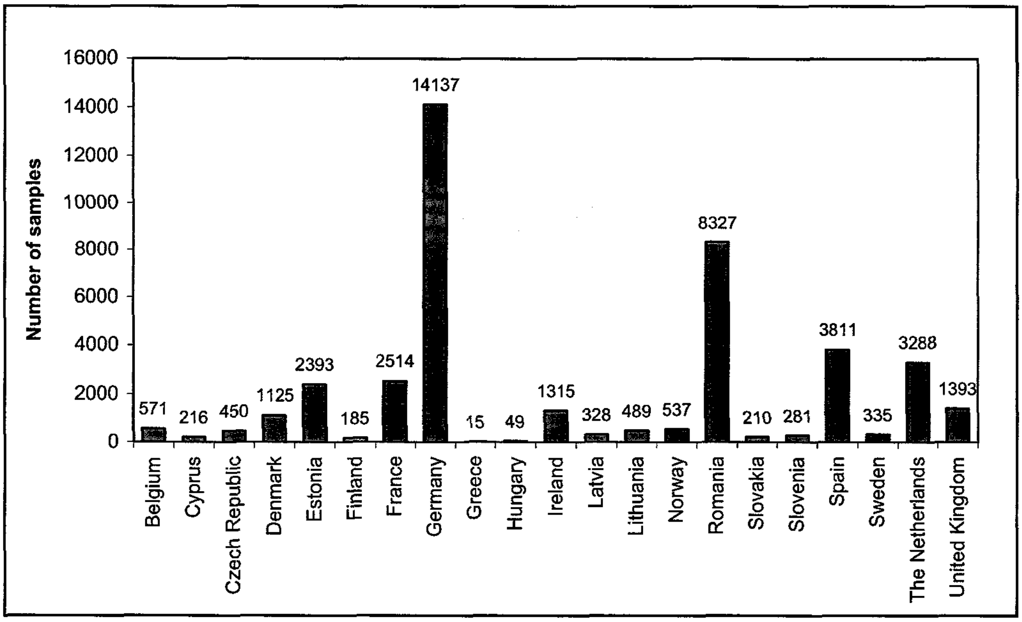

Figure 2. Number of results for vegetables submitted by each Member State.

Germany contributed almost 34% of the results, with Romania the second largest contributor at close to 20% and Spain the third largest at just over 9%. The country of origin of the vegetables was indicated for 72.3% of the data and varied considerably from the testing country (Figure 3). Vegetables imported from 37 third countries (excluding Norway) comprised only 1% of the data, with 98 samples from Morocco, 80 from Turkey, 58 from Israel and 49 from Egypt being the highest numbers.

There was a considerable flow of product across Member State borders. Examples of the submitted data with known country of origin showed Germany produced 63% of the vegetables within its borders and imported 12% from Italy, 7% from Spain, 6% from the Netherlands, 5% from Belgium, and another 7% from other countries. Denmark produced 74% of the vegetables within its borders and imported 7% from Italy and Spain, respectively, 4% from the Netherlands, 3% from Germany and a further 5% from other countries.

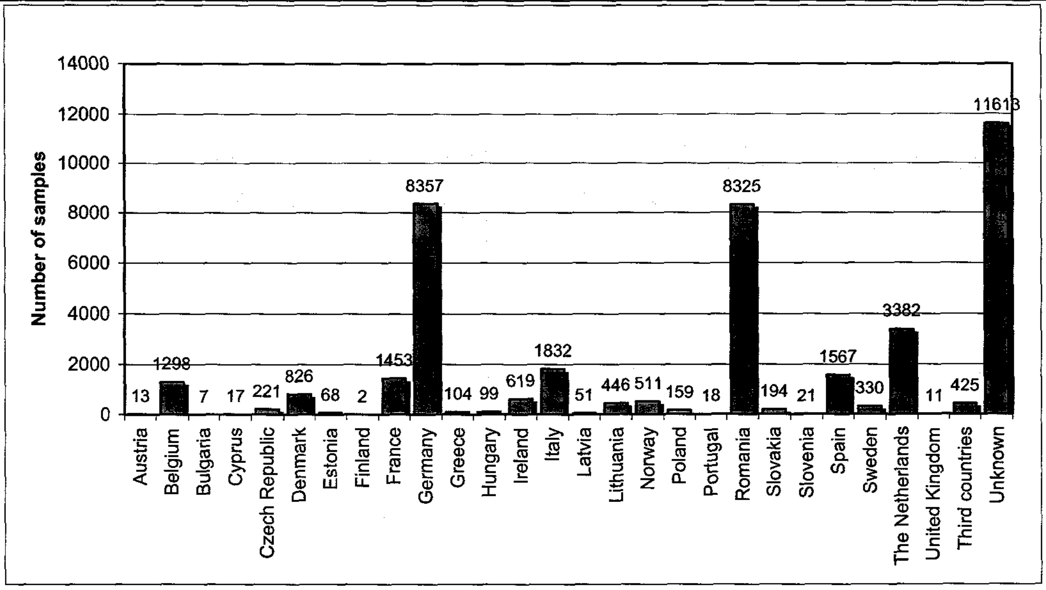

Figure 3. Country of origin data for vegetables.

Results reported covered 92 different vegetable varieties, although for 23 of those less than 10 samples of each had been tested comprising 60 in total. They have been excluded from the further analysis because of the uncertainty associated with isolated results. It is nevertheless worth mentioning that some leafy herbs like mint, oregano and thyme contained nitrate levels higher than 5,000 mg/kg, but would on the other hand be consumed only in low amounts. Less than 5% of all samples (1,934 sample results) were reported as below the limit of detection (LOD) for nitrate, and no further information was provided on how data were reported at the LOD. The LOD for the methods used to analyse these samples varied between 1 and 500 mg/kg as reported by the different laboratories, however 60% of these analytical results were reported with a LOD of 5 mg/kg or below and less than 2% with a LOD above 100 mg/kg.

Nitrate concentrations for vegetables where 10 or more samples have been analysed are presented in Tables 2 to 10 grouped into product categories as defined in regulation EC 178/2006[15] of 1 February 2006 for maximum levels of pesticide in food and feed. Statistical descriptors include median and mean concentrations as well as the 5th and 95th percentile concentrations (abbreviated as P5 and P95, respectively). Overall there are 41,415 datapoints, whereas 554 were excluded from the calculations as they comprised less than 10 samples or belonged to herbs and spices.

Vegetables were not proportionally tested to reflect their true part of the diet, but sampling disproportionately targeted species with maximum legislated limits. To correctly calculate the dietary nitrate impact of each species the relative proportion of each type of vegetable in the diet was estimated and used as a weighting factor calculated by means of dividing the estimated relative dietary proportion by the relative sample proportion, applied when calculating the overall median presented in Table 11. No such corrections were applied to the individual vegetable group calculations. It proved very difficult to get accurate consumption figures for all vegetable

[15] OJ L29 2.2.2006

varieties and production volumes were thus used as proxies for consumption. The GEMS/Food regional European cluster diet was used as a starting point with some broad categories further split to species level by applying European horticultural production statistics (WHO, 2003a; EuroStat, 2007). The calculated weighting factors applied for each species in 9 different vegetable groups are given in Tables 2 to 10. The weighted overall result for vegetables including roots and tubers, but excluding herbs, is given in Table 11.

Table 2. Nitrate concentrations in brassica vegetables.

Brassica vegetables	Number of samples (weighting factor)	Nitrate concentration (mg/kg)			
		P5	Median	Mean	P95
Broccoli	227 (*0.54)	16	209	279	758
Brussels sprouts	130 (*0.54)	1	1	24	100
Cabbage	1,198 (*3.99)	47	223	311	833
Cauliflower	289 (*2.06)	7	122	148	390
Chinese cabbage	469 (*0.02)	77	870	933	1,928
Curly kale	169 (*0.10)	19	267	537	1,846
Kohlrabi	135 (*0.02)	142	940	987	1,830
Red cabbage	196 (*0.60)	35	250	281	704
Sauerkraut	37 (*0.02)	37	42	66	215
Savoy cabbage	342 (*0.02)	1	204	324	1,144
Total	3,192	7	241	411	1,383

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Brussels sprouts had particularly low nitrate concentrations. Most of the vegetables in the brassica group had median nitrate concentrations of approximately 40 to 200 mg/kg except Chinese cabbage and kohlrabi with concentrations around 900 mg/kg. The maximum recorded level of 4,900 mg/kg originated from ordinary cabbage imported from China. A further 349 samples or 11% had values above 1,000 mg/kg.

Table 3. Nitrate concentrations in bulb vegetables.

Bulb vegetables	Number of samples (weighting factor)	Nitrate concentration (mg/kg)			
		P5	Median	Mean	P95
Garlic	13 (*0.60)	8	70	69	161
Onions	230 (*5.55)	1	60	164	638
Total	243	1	60	159	601

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Bulb vegetables were generally low in nitrate as shown in Table 3, although there were very few test results.

Table 4. Nitrate concentrations in fruiting vegetables.

Fruiting vegetables	Number of samples (weighting factor)	Nitrate concentration (mg/kg)			
		P5	Median	Mean	P95
Aubergine	182 (*0.46)	29	303	314	572
Capsicum	455 (*0.40)	1	28	108	476
Chili pepper	152 (*2.08)	4	52	67	120
Courgette	159 (*0.02)	11	297	416	1,060
Cucumber	898 (*0.90)	22	156	185	409
Gherkin	88 (*0.90)	11	40	69	230
Pumpkin	32 (*0.70)	8	392	894	4,617
Tomato	856 (*6.96)	1	26	43	144
Total	2,822	1	83	149	486

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

The fruiting vegetables group, although disparate, had the third lowest median concentration of nitrate after the legume and bulb vegetable groups (not counting the few samples in the fungi group). There were some surprisingly high concentrations reported for a few pumpkin samples with the maximum at 5,665 mg/kg found in France and a few courgette samples over 1,000 mg/kg, but most other samples were below this latter level.

Table 5. Nitrate concentrations in fungi.

Fungi	Number of samples (weighting factor)	Nitrate concentration (mg/kg)			
		P5	Median	Mean	P95
Mushroom	12 (*0.80)	31	43	61	100
Total	12	31	41	59	100

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Only a few sample results from fungi testing were reported, generally on the low side.

Table 6. Nitrate concentrations in herbs.

Herbs	Number of samples	Nitrate concentration (mg/kg)			
		P5	Median	Mean	P95
Basil	68	94	1,827	2,292	5,174
Borage	15	200	1,536	1,918	4,550
Chives	83	1	307	748	2,949
Coriander	20	1,135	2,468	2,445	3,982
Dill	57	13	1,123	1,332	4,294
Parsley	249	10	480	958	3,404
Total	492	10	791	1,240	4,040

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Several products belonging to the herbs group had high median nitrate concentrations and also the 95[th] percentile approached the concentration found in leafy vegetables. However, there is less concern in relation to this group since the volume consumed will only be small. It is therefore not included in the calculation of the overall vegetable nitrate statistics thus no weighting factors are given.

Table 7. Nitrate concentrations in leafy vegetables.

Leafy vegetables	**Number of samples (weighting factor)**	**Nitrate concentration (mg/kg)**			
		P5	**Median**	**Mean**	**P95**
Amaranth	12 (*0.01)	439	2,660	2,167	3,483
Beet	12 (*0.002)	84	1,770	1,852	3,685
Belgian endive	1,006 (*0.41)	63	1,475	1,465	3,063
Butterhead lettuce	3,426 (*1.44)	53	1,978	2,026	4,090
Cos lettuce	124 (*1.03)	167	1,097	1,105	2,200
Curled lettuce	301 (*0.10)	16	1,628	1,601	3,400
Dandelion	23 (*0.02)	5	202	605	2,747
Escarole	73 (*0.002)	6	298	523	1,579
Iceberg lettuce	1,980 (*2.06)	210	844	875	1,537
Lamb's lettuce	710 (*0.10)	121	2,130	2,104	3,833
Lettuce	7,749 (*2.06)	56	915	1,324	3,660
Mixed lettuce	89 (*2.01)	281	1,878	2,062	5,242
Oak-leaf lettuce	470 (*0.10)	8	1,553	1,534	3,285
Radicchio	40 (*0.10)	5	339	355	829
Rucola	1,943 (*0.10)	1,528	4,800	4,677	7,340
Silverbeet (chard)	666 (*0.10)	178	1,510	1,690	3,685
Spinach	6,657 (*0.52)	64	785	1,066	3,048
Water cress	25 (*0.02)	4	12	136	174
Total	25,306	66	1,140	1,614	4,556

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Leafy vegetables clearly had the highest median value of all groups. The highest nitrate value recorded in the group, 19,925 mg/kg, belonged to an oak-leaf lettuce sample grown under cover in Norway. However, rucola had the highest median concentration of nitrate (4,800mg/kg) of any vegetable with 56% of values over 4,500 mg/kg, followed by amaranth and Lamb's lettuce. Butterhead lettuce, a common salad vegetable, had a median nitrate concentration just below 2,000 mg/kg and 2% of the samples exceeded 4,500 mg/kg. The median for spinach at 785 mg/kg was well below the maximum level allowed with 5% of the samples exceeding 3,000 mg/kg.

Table 8. Nitrate concentrations in legumes.

Legumes	**Number of samples (weighting factor)**	**Nitrate concentration (mg/kg)**			
		P5	**Median**	**Mean**	**P95**
Beans	48 (*0.24)	6	435	392	810
French beans	52 (*0.24)	4	20	756	3,970
Green beans	362 (*2.39)	9	293	323	735
Peas	407 (*2.79)	1	1	30	100
String beans	13 (*0.24)	170	610	618	900
Total	882	1	56	221	748

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Legume results were generally fairly low except for 27% of the French bean results, which had nitrate concentrations over 1,000 mg/kg.

Table 9. Nitrate concentrations in stem vegetables.

Stem vegetables	Number of samples (weighting factor)	Nitrate concentration (mg/kg) P5	Median	Mean	P95
Asparagus	260 (*0.30)	1	24	209	1,459
Celery	387 (*0.40)	18	693	1,103	3,319
Fennel	116 (*0.002)	25	783	1,024	3,047
Leek	558 (*0.40)	5	257	345	975
Rhubarb	58 (*0.40)	28	2,808	2,943	6,550
Total	1,379	3	302	698	2,923

Samples below the LOD were expressed as upper bound value that which the actual LOD was used in the calculations.

The median for stem vegetables was higher than for fruiting vegetables as would be expected from plant physiology. Rhubarb showed particularly high values.

Table 10. Nitrate concentrations in roots and tubers.

Roots and Tubers	Number of samples (weighting factor)	Nitrate concentration (mg/kg) P5	Median	Mean	P95
Artichokes	65 (*1.10)	1	21	174	375
Beetroot	1,013 (*0.40)	110	1,100	1,379	3,670
Black radish	19 (*0.002)	233	1,245	1,271	2,302
Black salsify	12 (*0.002)	1	12	43	230
Carrot	2,383 (*4.39)	21	125	296	1,574
Celeriac	41 (*0.002)	20	263	390	975
Parsnip	22 (*0.40)	2	16	83	349
Potato	2,795 (*48.05)	10	106	168	340
Radish	788 (*0.40)	115	735	967	2,515
Turnip	241 (*0.40)	10	312	663	3,400
White radish	200 (*0.02)	135	1,256	1,416	3,488
Total	7,579	15	152	506	2,302

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Although the median concentration of nitrate for the roots and tuber group is low at 152 mg/kg, the median values for the different product groups ranged from 12 to 1,256 mg/kg. Potatoes and carrots are both major components in the diet of many countries and the medians are just above 100 mg/kg. Beetroot, on the other hand, is almost ten times higher at a median of 1,100 mg/kg, but is consumed much less frequently except in a few countries. The mean is more than three times higher than the median indicating some high values at the tail end of the distribution. This could influence local intake, particularly in self-production systems with little variation in source of produce. There are 64 results higher than 4,000 mg/kg with more than half belonging to beetroot and also turnip being over-represented. The samples with high concentrations were produced in seven countries with The Netherlands and to some extent Hungary having more samples with high concentrations than would be expected from the number of overall samples tested.

In summary, there is a large variation in median concentrations of nitrate in different vegetables from a low of 1 mg/kg (peas and Brussels sprouts) to a high of 4,800 mg/kg (rucola).

To avoid sampling bias when calculating an overall median for the vegetable group, results for each vegetable variety were adjusted according to approximated consumption volumes as estimated by production volumes. The weighted overall result for the nitrate content in vegetables including roots and tubers but excluding herbs was a median best estimate of 255 mg/kg (Table 11).

Table 11. Overall nitrate concentration used in the assessment of vegetables calculated by applying a weighting factor to individual results according to relative production volumes for the variety.

Overall vegetables	Number of samples	Nitrate concentration (mg/kg)			
		P5	Median	Mean	P95
All (including roots and tubers)	41,415	27	255	336	851

Samples below the LOD were expressed as upper bound value that means the actual LOD was used in the calculations.

Variation of nitrate levels due to geographical differences

Apart from the bias introduced by the number of samples analysed for each type of vegetable, uncertainty in the estimated overall concentration of nitrate in vegetables is also associated with the representativeness of geographical coverage and season, as well as the production and processing methods utilised.

Since there is a complex interaction between season, production method and location in relation to the amount of sunlight and the accumulation of nitrate in vegetables as has been described earlier, an attempt was made to disaggregate those factors. Countries were allocated to one of three regions:

- North including Finland, Sweden, Norway, Iceland, Denmark, the United Kingdom, Ireland, Estonia, Latvia and Lithuania;
- Central including Poland, Germany, The Netherlands, Belgium, Luxembourg, Czech Republic, Slovakia, Austria and Hungary; and
- South including France, Portugal, Spain, Italy, Malta, Greece, Slovenia, Rumania and Bulgaria.

Samples were also recorded as being produced during winter or summer and in open air or under cover.

Lettuce varieties were treated as a group including butterhead lettuce, cos lettuce, curled lettuce, iceberg lettuce, Lamb's lettuce, lettuce (unspecified), mixed lettuce and oak-leaf lettuce. Rucola and spinach were studied separately. A detailed analysis of the influence of season, production method and location was performed on those products. Samples lacking such details were omitted from the analysis. The lettuce varieties group was stratified using a modelling approach applying estimated market share as indicated above to the respective variety and curve fitting to calculate median and percentile values. A logistic equation was applied for all varieties as the best fit equation using @Risk (Palisade Corporation). Generally nitrate levels found in lettuce varieties produced in southern Europe were lower than levels found in central or northern Europe (Figure 4).

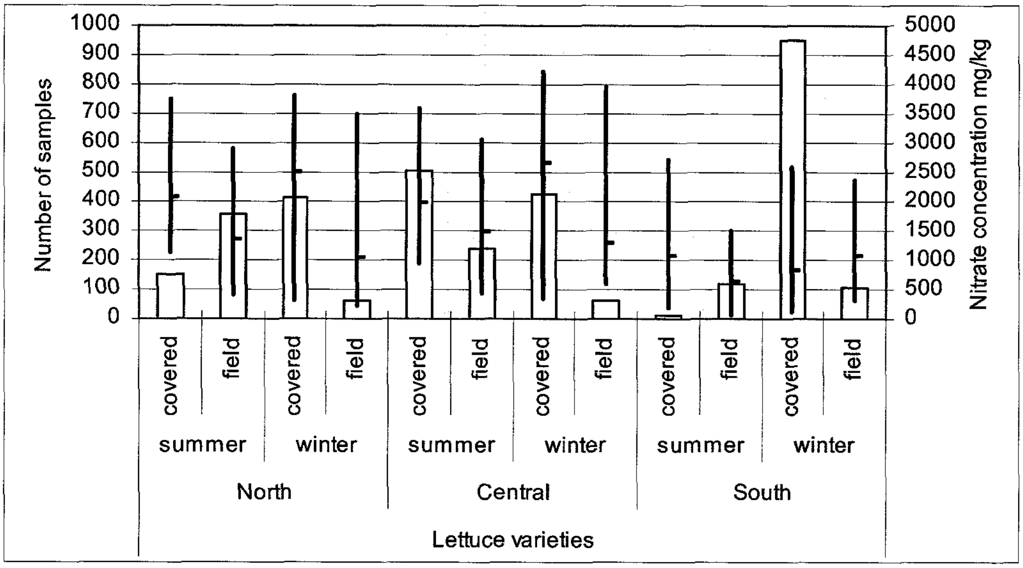

Figure 4. Levels of nitrate in lettuce varieties as influenced by season, production system and region. Thick bars illustrate the number of sample results (left y-axes) and thin bars the 5th, 50th (crossbar) and 95th percentile values in mg/kg (right y-axes).

Production under cover (potentially reduced light intensity) increased nitrate levels irrespective of season except for lettuce varieties produced in southern Europe during winter. Variations in median concentrations of nitrate in lettuce varieties across all production conditions of more than eight times were recorded, with the lowest of 625 mg/kg found in summer field production in the south and the highest of 2,652 mg/kg found in covered winter production in central Europe.

Rucola was a staple component of the Roman diet in ancient times. This is borne out by poets like Horace who credited his friend Martial, who spoke of it as a magical herb, with having discovered its aromatic and flavouring properties. However, its popularity decreased and it was almost absent in common diets for many centuries. Now the use of rucola as part of a leafy salad mix or by itself again seems to be increasing in several European diets. Currently rucola is used in cooking as an herb, a side dish to accompany meat dishes and as a topping for first courses. Rucola is grown in the open during the summer season, but is also grown in greenhouses during the winter season, which could result in increased nitrate levels. The nitrate levels of rucola as influenced by season and region are shown in Figure 5.

Variations in nitrate levels found in rucola under different production conditions were much less than for the lettuce varieties with a maximum 68% difference. However, sample numbers were low for most groups. Rucola is not captured by the current legislation limiting nitrate concentrations in some vegetables.

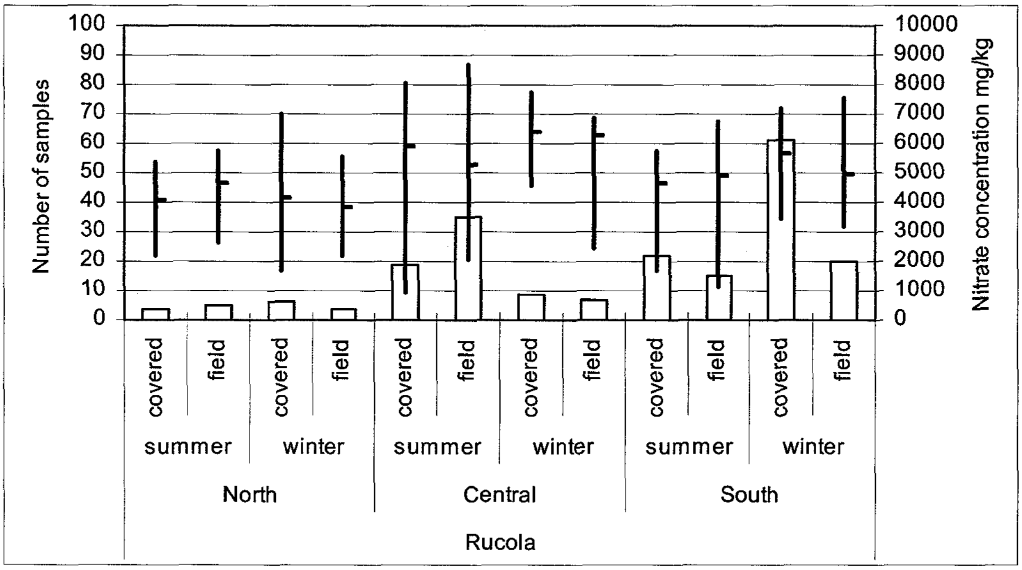

Figure 5. Levels of nitrate in rucola as influenced by season, production system and region. Thick bars illustrate the number of sample results (left y-axes) and thin bars the 5th, 50th (crossbar) and 95th percentile values in mg/kg (right y-axes).

Spinach has often been seen as a major vegetable source for nitrate in the diet and home made baby food including spinach stored under inappropriate conditions has been involved in some cases of methaemoglobinaemia, (Filer *et al.*, 1970; Sánchez-Echaniz and Benito-Fernández, 2001). Median levels of nitrate in spinach were mainly below 2,000 mg/kg (Figure 6).

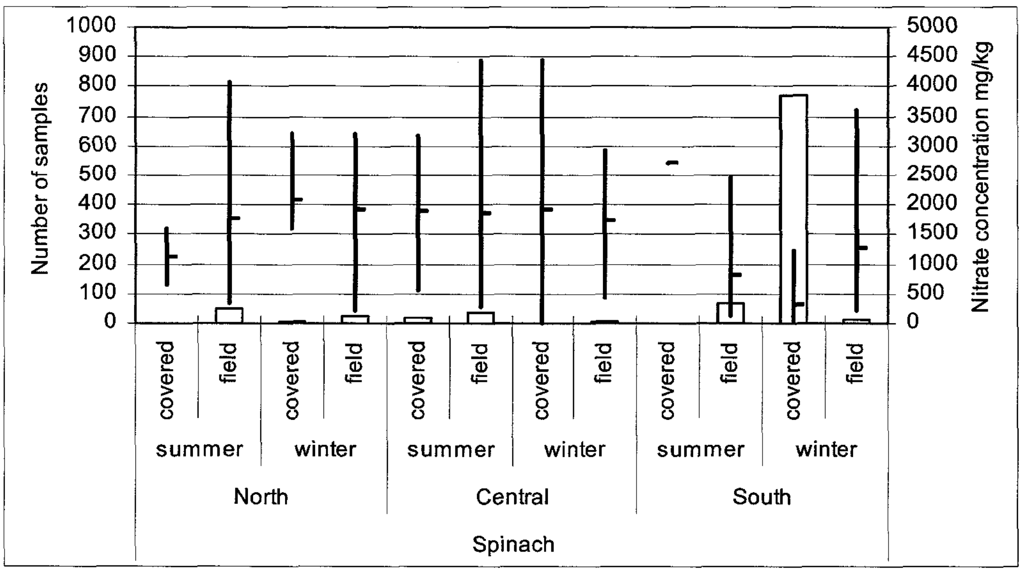

Figure 6. Levels of nitrate in spinach as influenced by season, production system and region. Thick bars illustrate the number of sample results (left y-axes) and thin bars the 5th, 50th (crossbar) and 95th percentile values in mg/kg (right y-axes).

It is of interest to note that production under cover of spinach during winter in southern Europe achieved the lowest levels of nitrate indicating that this is a well controlled system. The low number of sample results in the rest of the matrix does not allow further conclusions to be drawn.

Table 12 summarises the occurrence data for nitrate in vegetables and vegetable groups which are used in the exposure assessment. The median concentrations given for other vegetables and for lettuce varieties have been adjusted by applying a weighting factor calculated according to estimated volume of production/consumption for each vegetable.

Table 12. Median occurrence data for nitrate in vegetables and vegetable groups by applying a weighting factor to individual results according to relative production volumes for the variety. These values have been used in the exposure assessment. N= number of results reported.

	Nitrate concentration mg/kg				
	Most vegetables [a)]	Lettuce varieties	Spinach	Rucola	Potato
Median	392	1,338	785	4,800	106[b)]
Median range	-	625-2,652	386-1,745	3,805-6,400	-
N	33,836	14,849	6,657	1,943	2,795

a) All vegetable products including the also separately shown lettuce varieties, spinach and rucola but excluding roots and tubers, and herbs.

b) Potatoes representing roots and tubers.

The median range reflects the different production conditions as illustrated in Figures 4 - 6. The upper limits represent adverse growing conditions (less sunlight, under cover etc) or excess nitrogen fertilisation and can be a worst case scenario under local circumstances e.g., dominating the diet for some time if consuming self-produced product exclusively.

Nitrite information was not requested from Member States. Data from the literature (Jakszyn *et al.*, 2004) showed that vegetables in general contribute only approximately by 2 to 6% of daily dietary intake to the total nitrite exposure (including endogenous conversion from nitrate). This low amount is overwhelmed by the endogenous conversion of dietary nitrate from vegetable consumption to nitrite through entero-salivary recirculation. The direct nitrite intake from vegetables is thus well within the margins of error for the occurrence analysis and nitrite occurrence was not studied in detail.

5.1 Influence of storage and food processing on nitrate levels

Levels of nitrate and nitrite in raw agricultural commodities can be influenced by a number of factors such as storage time and conditions (i.e. ambient, refrigerated, frozen), and food processing (i.e. washing, peeling, blanching, boiling). Overall, there is a paucity of published data in this area.

5.1.1 Storage

Ambient temperature

Nitrate levels in raw vegetables kept at ambient temperatures can decrease during the period of storage. On the contrary, nitrite levels in fresh, undamaged plant tissues are usually very low but post-harvest storage and wilting processes favours its increase. The increase in nitrite levels may be dependent on species differences, specific endogenous nitrate reductase activities (Pate, 1973; Andrews, 1986; Wallace, 1986) and the amount of bacterial contamination (Phillips, 1968; Ezeagu and Fafunso, 1995; Ezeagu, 1996; Chung *et al.*, 2004).

Studies on nitrate and nitrite levels in spinach (Phillips, 1968; Chung *et al.*, 2004), Nigerian leaf vegetables (Ezeagu and Fafunso, 1995; Ezeagu, 1996) and Chinese cabbage (Chung *et al.*, 2004) under storage at ambient temperature indicated that nitrate content decreased whereas nitrite tended to increase over time. This process was accelerated when the produce was pureed.

Refrigerated

Under refrigerated storage (7 days) at 5°C, nitrate levels were almost unaffected in Chinese cabbage and spinach, respectively. Nitrite concentrations remained low over the whole storage period (Chung *et al.*, 2004). This implies inactivation of endogenous nitrate reductase under cold storage conditions as well as prevention of bacterial activity.

On the other hand, high levels of nitrite have been found in home-made vegetable purees even after refrigerated storage for only 12 hours or more (Sánchez-Echaniz and Benito-Fernández, 2001). Presumably pureeing releases endogenous nitrate reductase causing excessive formation of nitrite particularly in vegetables containing high level of nitrate like spinach and silverbeet. The authors recommended that infant food should be prepared for immediate use or kept frozen when consumption is delayed for more than 12 hours.

Frozen

Nitrite accumulation is inhibited under frozen storage (Phillips, 1968). Schuster and Lee found no significant changes in nitrate or nitrite content of spinach, beet, carrot, parsley-root, celery or potatoes during frozen storage for up to 12 weeks, (Schuster and Lee, 1987).

5.1.2 Processing

Washing

Nitrate is soluble in water and washing of leafy vegetables (lettuce, Lamb's lettuce, endives) can reduce nitrate levels by 10-15% (Dejonckheere *et al.,* 1994). Mozolewski and Smoczynski (2004) showed that levels of nitrate and nitrite in potatoes can also be decreased by 18 to 40 % and 25 to 75%, respectively after preliminary processing methods (washing, peeling and rinsing).

These findings are in line with other studies (Czarniecka-Skubina and Golaszewska, 2001; Golaszewska and Zalewski, 2001).

Peeling

The nitrate content in two potato varieties (Innowator and Santana) before peeling was 258 and 349 mg/kg dry matter, respectively, and it decreased considerably during French fries production. About 30% of the nitrate was removed during peeling. Preheating and cutting reduced the nitrate content by a further 20% and blanching by 30%. After final frying only 5-6% of the original nitrate content remained or 16-18 mg/kg dry matter (Rytel *et al.*, 2005).

After peeling of potatoes, bananas and melons the nitrate content decreased by 34%, 62% and 41% (Dejonckheere *et al.*, 1994) and only by 20% or 6.6% in beetroots for nitrate and nitrite, respectively (Czarniecka-Skubina *et al.,* 2003).

Cooking

The distribution of nitrate in vegetables is not even across the product. For lettuce and spinach elimination of the stem and midrib resulted in a decrease of the nitrate content of 30-40% (Dejonckheere *et al.,* 1994). The "flesh" makes up the bulk of the carrot, but had a significantly lower concentration of nitrate than the core tissue (Schuster and Lee, 1987). The largest amount of nitrate in potatoes is found in and just under the skin, however nitrite is more evenly distributed (Marin *et al.*, 1998).

Different studies have shown reduction of nitrate levels when vegetables are cooked in water. Peas, cabbage, beans, carrots, potatoes and spinach, endives and celery leaves lost between 16 to 79%, of the nitrate, respectively, during cooking (Abo Bakr *et al.*, 1986, Schuster and Lee, 1987, Dejonckeere *et al.*, 1994). The content of nitrate and nitrite decreased similarly after boiling by about 50% in carrot, parsley-root, celery and potatoes (Roszczenko *et al.*, 2001). Varoquax *et al.* (1986) showed that the diffusion of nitrate from carrots depended on water temperature, surface area (thickness of the carrot slice) and ratio of carrot to water with the total content of nitrate as measured in the carrot and water combined remaining constant (Schuster and Lee, 1987).

During thermal processing of potato tubers with different heating methods (boiling, microwave, steaming, and deep frying) losses of nitrate (16-62%) and nitrite (61-98%) have been reported (Mozolewski and Smoczynski, 2004). The greatest decrease in reducing nitrate (36-58%) and nitrite (82-98%) was observed when peeled potatoes were boiled in water compared to steaming methods. Deep frying of potatoes resulted also in considerable losses of nitrate (50-62%). However other studies reported that frying and baking of potatoes did not affect nitrate concentrations (MAFF, 1998a,b). Overall the losses of nitrite were greater then for nitrate when applying different preliminary processing and heating methods. Differences in nitrate and nitrite

losses were observed between potato varieties subjected to the same processing conditions (Mozolewski and Smoczynski, 2004).

Other ways of food processing

Limited data are available on nitrate and nitrite levels in canned vegetables. One study found that canned vegetables contained much higher amounts of nitrite (450 mg/kg) than those reported in the raw commodity (Jakszyn, *et al.,* 2004).

In red beet and kohlrabi, nitrate was reduced by fermentation by up to 50% and in white cabbage by up to 87% (Preiss *et al.*, 2002).

In summary, handling, storage, processing including washing, peeling and cooking can significantly reduce the amount of nitrate in vegetables. This holds true for vegetables eaten cooked, like potato, spinach and cabbage. For vegetables eaten raw, only handling and storage would impact nitrate levels. Since there is a trend nowadays towards consumption of fresh produce, and in particular leafy vegetable varieties, a conservative approach was adopted. Thus, the potential decreases in nitrate concentrations due to processing were not considered for the initial exposure calculations but can be considered as mitigating factors in a range of mixed vegetable consumption scenarios.

6. Consumption of vegetables

The World Health Organisation is responsible for the Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme, commonly known as GEMS/Food. As part of its mandate to assess the potential exposure of populations to chemicals in food, GEMS/Food from 1989 onwards produced estimates of regional dietary patterns of raw and semi-processed food commodities. The latest GEMS/Food European Regional Diet from 2003 was previously discussed when stratifying occurrence data in Chapter 5 since it provides a Europe wide estimate (WHO, 2003a). In 1997, GEMS/Food was given the mandate to refine the regional diets and in 2006 introduced the GEMS/Food Consumption Cluster Diets database (WHO, 2006). The GEMS/Food Consumption Cluster Diets are based on national food balance sheets of annual food production as well as import and export for individual countries aggregated into clusters according to similar consumption behaviour. The main advantage of the data is the good comparability between different countries because the same methodology and standardised food classification system of the Codex Alimentarius were used. There are 13 cluster diets in total and because different EU Member States are part of four clusters (Table 13) there are some indications of variability in intake patterns. However, data from food balance sheets do not give information on consumption at the individual level, so that only a "per capita" mean consumption amount of a population can be derived. Information on high percentiles of the population and on selected population subgroups (age-groups, vulnerable subgroups) cannot be derived from these data.

Table 13. Composition of GEMS/Food Consumption Cluster Diets that include European Member States and Norway.

Cluster B	Cluster D	Cluster E	Cluster F
Cyprus	Albania	Austria	Estonia
Greece	Armenia	Belgium	Finland
Israel	Azerbaijan	Croatia	Iceland
Italy	Belarus	Czech Republic	Latvia
Lebanon	Bosnia and Herzegovina	Denmark	Lithuania
Portugal	Bulgaria	France	Norway
Spain	Georgia	Germany	Sweden
Turkey	Iran, Islamic Rep of	Hungary	
United Arab Emirates	Kazakhstan	Ireland	
	Kyrgyzstan	Luxembourg	
	Moldova, Republic of	Malta	
	Romania	Netherlands	
	Russian Federation	Poland	
	Serbia and Montenegro	Slovakia	
	Tajikistan	Slovenia	
	The former Yugoslav Republic of Macedonia	Switzerland	
	Turkmenistan	United Kingdom	
	Ukraine		
	Uzbekistan		

Consumption of vegetables in the four cluster diets including EU Member States is illustrated in Table 14 as well as the GEMS/Food European regional diet.

Table 14. Consumption of vegetables as estimated in the GEMS/Food Consumption Cluster Diets for the four clusters relevant to the EU and the European Regional Diet.

Food Group	GEMS/Food - consumption g/person per day				
	Cluster diets				Regional diet
	B	D	E	F	Europe
Roots and tubers	246	244	277	205	242
Other vegetables	525	267	249	197	372

Consumption of roots and tubers are fairly similar between the different diets. However, there is considerable variation in overall vegetable intake with the cluster B estimated other vegetable consumption more than double the amount of cluster F. The average European regional diet of 372 g seems to be a good overall approximation of an average of the four cluster diets for other vegetable consumption.

Consumption information was also supplied by 11 Member States and Norway including different ranges of vegetables depending on the data available as shown in Table 15. The information was extracted from the most recent national food consumption surveys undertaken within the last ten years. Different survey methodologies used make a direct comparison between countries unreliable, but the information can be used to identify high consumers.

Table 15. Individual vegetable consumption information as supplied by MS and Norway.

Product	Country	Consumption g/person/day Whole population			Eaters only			%
		Mean	P95	P97.5	Mean	P95	P97.5	
Broccoli	Germany	4	19	22	11	24	33	39
	Ireland	6	24	34	13	36	46	43
Cabbage	Czech Republic	23						
	Estonia	25						
	Germany	45	106	128	46	107	129	97
	Ireland	11	39	46	18	41	53	64
	Norway	4	16	22	6	19	29	58
	Spain	2	18	21	21	59	66	10
	Sweden	5			14			33
Cauliflower	Czech Republic	7						
	Estonia	1						
	Germany	11	33	42	14	36	46	80
	Ireland	3	19	26	12	28	39	27
	Norway	1	3	7	4	17	19	14
	Spain	4	30	35	47	101	112	8
Celery	Czech Republic	4						
	Germany	0	0	1	4	11	19	3
	Ireland	1	6	9	3	10	16	39
	Spain	0	5	6	12	35	39	2
Chard leaves	Spain	5	36	42	55	108	119	9
Ch. cabbage	Norway	4	15	20	5	15	22	79
Courgette	Ireland	1	4	8	7	23	25	8
	Spain	3	25	30	31	76	84	11
Cucumber	Czech Republic	18						
	Estonia	19						
	Ireland	1	9	16	7	25	28	20
	Norway	6	22	31	7	23	34	79
	Spain	2	16	19	17	47	53	12
	Sweden	9			12			70
Curly kale	Germany	1	9	17	12	30	34	11
Green onion	Czech Republic	25						
	Estonia	16						
	Ireland	16	42	48	16	42	49	97
	Norway	0	0	1	0	1	1	39
	Spain	13	40	45	18	45	50	72
Iceberg lettuce	Germany	1	2	10	11	37	51	7
	Ireland	2	10	14	6	20	24	28
Lettuce	Czech Republic	4						
	Germany	17	63	79	23	71	92	71
	Ireland	1	8	11	5	15	21	24
	Spain	36	103	116	57	120	133	62
Parsley	Czech Republic	2						
	Germany	1	3	3	1	3	3	99
Potato	Cyprus	144						
	Czech Republic	199						
	Estonia	224						
	Germany	119	244	280	121	244	280	99
	Ireland	247	608	768	249	610	771	100
	Lithuania	243						
	Spain	57	143	160	70	152	167	82
	Sweden	142			144			99

Table 15. continued.

Product	Country	Consumption g/person/day						%
		Whole population			Eaters only			
		Mean	P95	P97.5	Mean	P95	P97.5	
Spinach	Czech Republic	2						
	Ireland	0	0	4	8	16	24	4
	Spain	5	37	43	52	117	129	9
	Sweden	1			11			9
	UK	11		44				

P95 and P97.5 indicate the 95^{th} and 97.5^{th} percentile of consumption, respectively. Consumption amounts are given distributed across the whole population and across eaters only. The proportion of eaters is indicated in the last column and is a measure of consumers of the respective product during the survey period. Not all of the 11 Member States provided a full breakdown of the consumption statistics.

The information supplied was aggregated at different levels by Member States as shown in Table 16. The aggregated information is an approximation only. Some Member States aggregated their information into a group called leaf and stem vegetables, others into all lettuce or salad vegetables. There are considerable overlaps between those groups and the information should be taken as an indication only of the actual consumption.

The "most vegetable" grouping excludes potato consumption. The custom of including or excluding potato consumption when calculating total vegetable intake vary between regions.

The "eaters' only" frequency for other vegetables in Table 16 indicates consumption of at least one vegetable during the survey period explaining the almost 100% frequency.

Comparing the information provided through GEMS/Food based on vegetable volumes for production and trade with the individual Member State information based on reported consumption it is clear that as expected the GEMS/Food data is on the high side since it does not include wastage. Using the GEMS/Food information as an upper limit for mean consumption would thus prove to be a conservative approach.

Table 16. Aggregated vegetable consumption information as supplied by Member States and Norway.

	Consumption g/person/day						%
	Whole population			Eaters only			
	Mean	P95	P97.5	Mean	P95	P97.5	
Most vegetables							
Austria	148						
Czech Republic	213						
Lithuania	136						
Norway	135	317	393	136	318	393	99
Sweden	99			100			99
Leaf & stem vegetables							
Cyprus	64						
Germany	7	28	38	14	38	46	54
Lithuania	4			115			4
Sweden	17			25			69
All lettuce							
Belgium	8			23			36
Norway	4	15	20	5	15	22	79
UK	10		37				
Salad vegetables							
Germany	20	71	97	27	79	104	76
Cabbages							
Cyprus	58						
Sweden	3			12			22
Herbs							
Estonia	2						

Summarising the information the following can be noted:

- For "most vegetables", excluding potato, the highest 97.5th percentile daily consumption of 393 g per person was recorded in Norway (Table 16) and this was also close to the food balance information established by the GEMS/Food Regional Diet for a mean of 372 g per capita. A minimum amount of 400 g is also recommended by WHO for fruit and vegetable intake combined, which could be satisfied by consuming the whole amount as vegetables. In consequence and taking a conservative approach a daily consumption figure for "most vegetables" of 400 g was used in this opinion as the recommended target figure (exposure scenario S1, see chapter 7).

- For potatoes the highest 97.5th percentile daily consumption of 771 g per person was recorded in Ireland (see Table 15). This was almost three times higher than the mean recorded in the GEMS/Food Regional Diet or Cluster Diets. To estimate the upper limit for the potential contribution of potato consumption to nitrate exposure a figure of 771 g was used in a separate exposure scenario (S2).

- For leafy vegetables (lettuce) the highest 97.5th percentile daily consumption of 133 g per person was recorded in Spain (Table 15). To estimate the upper limit for the potential contribution of leafy vegetable consumption to nitrate exposure the figure of 133 g was used

for one scenario as the only vegetable and in a further scenario combined with the remainder of the recommended target case consumption allocated to other vegetables.

7. Exposure scenarios

The following exposure scenarios only include nitrate intake from vegetable sources. On the basis of the data collected, scenarios combining levels of consumption with concentration of nitrate in the relevant food category were elaborated. In summary, the base case assumed the consumption of vegetables, other than potatoes, at a level compatible with international dietary recommendations (400g/day) where the whole recommended intake was in the form of vegetables (no fruit). This represents a conservative approach. The influence of potatoes on the dietary exposure to nitrate was estimated in a separate scenario by using high percentile consumption. Since leafy vegetables exhibited high nitrate levels in general, high percentile consumption of leafy vegetables was applied to either spinach or "lettuce varieties" (Table 12) in two separate sub-scenarios, A and B respectively, see below. Those two sub-scenarios were expanded by also including the contribution of other vegetables using the remaining dietary intake from the base case. The impact of the trend to include rucola as a significant component of a leafy salad mix or by itself as a salad side dish or as a main pizza topping was tested in sub-scenario C. Finally, the highest recorded regional median concentrations of nitrate in spinach and "lettuce varieties" were applied to the previous sub-scenarios A and B to provide an upper estimate of nitrate dietary exposure. In calculating high percentile consumption, information from the GEMS/Food database or data submitted by the Member States were considered. The following presents a detailed description of the scenarios (S) used.

- S1 ("recommended vegetable and fruit intake scenario"): The first scenario is a base case founded on the daily consumption of 400 g of fruits and vegetables recommended by WHO to reduce the risk of coronary heart disease, stroke, high blood pressure and cancer (WHO, 2003b), but with the whole amount allocated to vegetables. This figure also corresponds very closely to the highest consumption of "most vegetables" reported by Norway as the 97.5th percentile of the distribution (Table 16) as well as GEMS/Food Regional Diet indicating a mean intake of 372 g per capita (Table 14). The concentration of nitrate used is the median of 392 mg/kg for "most vegetables" as presented in Table 12. It should be noted that some vegetables will be consumed only once a day or less while others might be consumed on several occasions. Because of the potential for chronic effects of nitrate, use of the median for "most other vegetables" stratified according to volumes consumed should cater for such variations.
- S2 ("potato scenario"): The second scenario was developed to separately determine the potential influence of potato consumption on nitrate intake at the upper level. It is based on the highest 97.5th percentile of consumption of potatoes of 771 g reported by Ireland (Table 15). The concentration of nitrate is the median for potatoes (Table 12). The result can be added to other scenarios, although it is considered unlikely that at this consumption level there is potential for further vegetable consumption.
- S3 ("green/leafy vegetables scenario"): The third scenario explores the influence of consumption of vegetables only from the "leafy vegetable" group containing the highest levels of nitrate recorded. It is based on the highest 97.5th percentile for consumption of

"leafy vegetables" (lettuce) of 133 g reported by Spain (Table 15). The median concentrations of nitrate in spinach and all combined lettuce varieties are used alternatively to provide respective scenarios S3A and S3B. In scenario S3C rucola is assumed to comprise one third of a leafy vegetable mix with lettuce varieties as the remaining two thirds.

- S4 ("combined S1 and S3 scenario"): In the fourth scenario it is assumed that while consuming leafy vegetables at the highest percentile volume as in scenario S3, other vegetables will be consumed as well. It is thus based on the recommended daily consumption of 400 grams as outlined in S1 by splitting consumption into 133 g of leafy vegetables, alternatively with the median concentrations of nitrate in spinach and lettuce varieties, and 267 g of other vegetables with their median concentration of nitrate to provide respectively scenarios S4A and S4B.
- S5 ("regional scenario"): The fifth scenario is similar to the fourth but the overall EU median concentration for leafy vegetables was replaced by the highest "regional" median concentration reported to estimate the impact of regional differences.

The main potential adverse effect of nitrate results from long-term exposure, and therefore the median or mean value is used for dietary exposure assessments. All the scenarios are based on median content of nitrate assuming that a consumer is randomly choosing vegetables on the market. Nevertheless the fifth scenario (S5) assumes that a consumer is choosing randomly vegetables from a "regional" market in which the median concentration for nitrate is higher than the overall European median concentration for the same categories of vegetables.

Results from calculating dietary exposure to nitrate in the different scenarios using a deterministic approach are presented in Table 17.

Table 17. Various dietary exposure scenarios based on nitrate from vegetables only.

	Vegetable consumption g/person/day	Vegetable	Overall median (S5 - highest regional median) nitrate concentration mg/kg [a]					Calculated exposure mg/person/day		
			Potato	Spinach	Lettuce	Rucola	Other	A	B	C
S1	400	Most					392	157		
S2	771	Potato	106					82		
S3	133 (44/89)[e]	Leafy		785	1,338	4,800		104[c]	178[d]	330[e]
S4	133/267	Leafy/Most		785	1,338		392	209[f]	283[g]	
S5	133/267	Leafy/Most		1,745[b]	2,652[b]		392	337[f]	457[g]	

a) See Table 12
b) Highest regional median
c) Spinach at 133 g
d) Lettuce varieties at 133 g
e) 1/3 of a leafy vegetable mix as rucola (44 g) and 2/3 as lettuce varieties (89 g)
f) Spinach at 133 g and other vegetables at 267 g
g) Lettuce varieties at 133 g and other vegetables at 267 g

A= spinach
B= all combined lettuce varieties
C= a mix of rucola (1/3) and lettuce varieties (2/3)

Table 17 indicates that consuming vegetables only at the levels found in many dietary recommendations for the combined consumption of vegetables and fruit (S1) could result in a nitrate exposure of 157 mg/person/day. A focus on potato consumption could for a high percentile consumer (S2) lead to nitrate exposure of 82 mg/person/day at the most. A high-level

consumer of either spinach (S3A) or lettuce varieties (S3B) could record nitrate exposure levels of 104 and 178 mg/person/day, respectively. However, by replacing a third of the lettuce varieties by rucola (S3C) the nitrate exposure would jump to 330 mg/person/day or almost double the contribution of lettuce varieties alone. In the event that the consumer from scenario S1 consumed spinach or lettuce varieties at the 97.5th percentile level as part of the 400 g vegetable mix, a third of the vegetables would be consumed as spinach (S4A) or lettuce (S4B) and the nitrate exposure would increase to 209 and 283 mg/person/day, respectively. Finally, should the last two scenarios occur in regions reporting the highest median nitrate occurrence levels, a nitrate exposure of 337 and 457 mg/person/day for spinach (S5A) and lettuce varieties (S5B), respectively, would be possible. However, only a very small proportion of the European population would even theoretically reach such level, it would be for sporadic periods only, and a number of mitigating factors would generally apply.

The various exposure scenarios demonstrated that the critical driver for a high dietary exposure to nitrate is not the absolute amount of vegetables consumed but the type of vegetable (i.e. leafy vegetables) and the concentration of nitrate related to the conditions of production. Thus consumption of a variety of vegetables, as promoted in dietary recommendations, contributed to less nitrate than lettuce varieties at an almost three times lower consumption level. Leafy vegetables grown under less favourable conditions had the potential to increase nitrate dietary exposure by 50-60%. The contribution of potato consumption to nitrate dietary exposure is lower or much lower than the contribution from a mix of other vegetables.

The above scenarios used nitrate concentrations as determined at retail for fresh commodities. Very few analytical results were available for nitrate in vegetables ready to eat. Washing of vegetables and heating have been shown to reduce nitrate concentrations to varying extents. A 40% reduction during cooking as indicated in a previous section would alone reduce the dietary nitrate intake in scenario S4B from 283 to 241 mg per day. A further mitigating factor is the role of fruit as part of fruit and vegetable consumption. Across 14 European countries examined, fruit contributed from a third to slightly more than half of the total fruit and vegetable consumption (EFSA, 2008). Fruit in general contains low levels of nitrate of the order of 10 mg/kg according to a review by White (1975) of three previous reports. Thus, a mixed fruit and vegetable diet can be estimated to reduce the base case scenario (S1) from 157 mg nitrate per day to between 81-106 mg with an estimated substitution of vegetables for fruit of 133-200 g of the 400 g daily total consumption.

The potentially high levels seen during winter in leafy vegetables in certain regions would not be sustained during the summer months. Thus, applying and combining the above mitigating factors that may occur from processing losses together with typical levels of fruit consumption as well as a six-month winter influence only, scenario S5A would be reduced to 171 mg per day and scenario S5B to 210 mg per day on an annualised basis.

Population subgroups

Accurate data are not available for children's fruit and vegetable consumption although it is known that overall they significantly favour the fruit component (Gregory *et al.* 2000; Richter *et al.*, 2008). Considering that nutritional recommendations are also valid for children, and in the absence of actual data the estimate was made that children could consume half the amount of adults. Thus 200 g of vegetables was considered to be a reasonable figure for children high consumers. Therefore, taking a body weight of 20 kg, the daily nitrate exposure for children would range from 2 to 12 mg/kg b.w./day under the different scenarios when calculating dietary intakes at half the adult levels and without considering mitigation factors.

Vegetarians and vegans are estimated to make up to 5% of the population of different Member States. Vegetarians might be suspected to have higher nitrate intakes than the general population. However, due to the physiological requirement for proteins, products from animal origin are likely to be substituted by cereals, nuts and pulses, generally low in nitrate, and not with excessive amounts of vegetables. Therefore, vegetarians and vegans are not considered likely to exceed significantly the 400g vegetables and fruit scenario. This was confirmed in a study conducted by the UK Ministry of Agriculture, Fisheries and Food (MAFF, 2000) where mean dietary exposure of vegetarians to nitrate was 83 mg/day and the highest 209 mg/day.

Nitrite contribution

Regarding nitrite, the evidence shows that actual nitrite levels in vegetables are not a major direct contributor to human exposure (see Figure 1 c, f). Similarly to nitrate, the dietary exposure should be based on the central tendency of the distribution of nitrite in vegetables (i.e. the median or the mean). Nitrite levels up to 45 mg/kg crop have been reported occasionally in the literature (Jakszyn *et al.*, 2004). A mean concentration of 0.5 mg/kg was found in the United Kingdom's 1997 total diet study (MAFF, 1998b) for all vegetables. Combining those 2 figures with the recommended amount of vegetables (400 g/day) results in a dietary exposure ranging from 0.2 to 0.8 mg/day corresponding to 0.003 to 0.013 mg /kg b.w./day assuming 60 kg body weight. This is low compared to systemic amounts of nitrite resulting from the bioconversion of nitrate (see Figure 1 c, f).

8. Hazard identification and characterisation

This section presents a historical perspective and a summary, mainly of human data, for both nitrate and nitrite as other data principally from animals have recently been subject to a detailed review by the JECFA (FAO/WHO, 2003a,b). Where new studies have been published these are included together with the existing core safety studies.

8.1 Toxicokinetics

The fate of nitrate has been the subject of a great number of studies and the results have been compiled in several reviews (e.g. FAO/WHO, 2003a). Nitrate undergoes a number of metabolic

interconversions, and is recycled between the saliva and the gut and the bile and the gut. Lately appreciation of the complexity of its metabolic handling has increased rapidly as the research area of nitric oxide physiology has expanded (e.g. Gladwin *et al.*, 2005).

Absorption

Nitrate

Nitrate is quickly and effectively absorbed from the upper part of the small intestine in humans after ingestion in food or water (Bartholomew and Hill, 1984; Ellen *et al.*, 1982; Spiegelhalder *et al.*, 1976; Turek *et al.*, 1980). For example, no or very little nitrate or nitrite is found in ileostomic fluid from persons who have ingested 250 mg of nitrate, suggesting that nitrate does not reach the large intestine (Ellen and Schuller, 1983). In humans, an average 25-fold increase in plasma nitrate was found 10 min after ingestion of nitrate, and intake peaked in blood after 40 min (Cortas and Wakid, 1991). In the rat, more than 50% of an oral dose was detected in the eviscerated carcass within 1 h (cited in Walker, 1990). In humans and most laboratory animals plasma nitrate is selectively absorbed by the salivary glands and concentrated 10-fold, resulting in a salivary secretion that represents approximately 25% of the ingested dose (Witter and Balish, 1979; Fritsch *et al.*, 1985). In humans the dose-dependent increase in salivary nitrate secretion, peaking 1-3 hours after oral ingestion (Bartholomew and Hill, 1984), is mediated by an active transport system that is shared also by iodide and thiocyanate (Forman *et al.*, 1985a,b).

Nitrate can also be absorbed via inhalation e.g. from cigarette smoke and car exhausts (Ellen and Schuller, 1983; Lundberg *et al.*, 2004 and 2008) although in absolute terms the quantitative amount is of minor importance compared to the oral route via the diet.

Nitrite

In humans, gastrointestinal (GI) absorption of sodium nitrite is rapid, with maximum plasma nitrite concentrations observed 15-30 min after dosing. Moreover, nitrite disappeared rapidly from plasma, with an average elimination half-life of 30 min. It was concluded that under fasting conditions 90-95% of orally administered sodium nitrite is absorbed from the gastrointestinal tract (Kortboyer *et al.*, 1997). However, extensive pre-systemic metabolism in the GI tract, results in a considerable part of the nitrite that enters the GI tract potentially being transformed to other N-containing species before absorption takes place (Speijers *et al.*, 1987).

Distribution

Nitrate

Absorbed nitrate is rapidly transported by the blood and selectively secreted by the salivary glands, and probably other exocrine glands, resulting in high salivary nitrate levels. After intravenous administration of ^{15}N-labelled nitrate in one volunteer, the labelled compound was rapidly distributed in the bloodstream throughout the body. The radioactivity accumulated almost

linearly with time in a small region of the abdomen, which probably was due to the swallowing and entero-salivary recirculation of nitrate/nitrite (Witter *et al.*, 1979).

Nitrite

Plasma nitrite levels are normally much lower than nitrate levels, firstly because of the lower exposure and secondly due to the rapid oxidation from nitrite to nitrate by oxygenated haemoglobin in the blood. Therefore, the sum of nitrate and nitrite in blood is almost identical to the nitrate levels (Lundberg and Weitzberg, 2005). This is also seen in body fluids and tissues of laboratory animals, where nitrite in the normal situation is practically absent, except in saliva where it increases as nitrate levels decrease (Witter and Balish, 1979; Fritsch *et al.*, 1985; Cortas and Wakid, 1991). In mice and rabbits, intravenous injection of labelled nitrite resulted in a homogenous distribution of radioactivity to a number of organs, including liver, kidneys and urinary bladder (Parks *et al.*, 1981).

Metabolism

There are some species differences in the metabolism of nitrate. In the case of humans, dogs and mini-pigs nitrate is concentrated from the plasma to the saliva and then commensal bacteria present on the back of the tongue reduce approximately 20% of the secreted nitrate to nitrite, which is then swallowed into the stomach. Nitrate is also secreted in the gut (Fritsch *et al.*, 1985; McKnight *et al.*, 1999, Xia *et al.*, 2003).

Although, *in vitro* studies with rat tongue section have shown that nitrate reduction occurs in the back of the tongue with Staphylococcus sciuri and Staphylococcus intermedius as the major nitrate reducing bacterial species (Li *et al.*, 1997). In the rat, oral reduction of nitrate to nitrite in the saliva is limited and nitrate is mainly secreted in the gastric and intestinal fluid by active transport involving entero-systemic recirculation as observed in man (McKnight *et al.*, 1999; FAO/WHO., 2003a; Mensinga *et al.*, 2003).

In humans, about 25 % of ingested nitrate is secreted in the saliva and approximately 20% of the secreted salivary nitrate is then converted to nitrite by microorganisms on the tongue and thus for normal individuals about 5-7% of ingested nitrate can be detected as salivary nitrite. However, for individuals with a high rate of conversion this figure may be up to 20% (Eisenbrand *et al.*, 1980; Speijers *et al.*, 1987; Kortboyer *et al.*, 1995; Lundberg *et al.*, 1994; FAO/WHO, 2003a). The major site for nitrate reduction is at the base of the tongue where a stable, nitrate-reducing microflora is present (McKnight *et al.*, 1999, Duncan *et al.*, 1995). The concentration of salivary nitrite is directly related to orally ingested nitrate (Stephany and Schuller, 1978; Spiegelhalder *et al.*, 1976), but the conversion may become saturated at high nitrate intakes (Tannenbaum *et al.*, 1976). Oral reduction of nitrate is the most important source of nitrite for humans, and will account for approximately 70-80 % of the human total nitrite exposure (Stephany and Schuller, 1980; Bos *et al.*, 1985). Factors that may influence the oral microbial flora are, e.g., nutritional status, infection, environmental temperature and age. Salivary nitrite levels were generally higher in older age groups, although considerable variation between individuals was noted (Eisenbrand

et al., 1980; Forman *et al.*, 1985a,b). Other factors such as antibacterial mouth wash may markedly lower the transformation of nitrate to nitrite (van Maanen *et al.*, 1998).

After transport to the stomach, the acidic conditions will rapidly transform nitrite to nitrous acid, which in turn will spontaneously decompose to nitrogen oxides including nitric oxide. Compared to the enzymatically produced nitric oxide in mammalian cells (from L-arginine by nitric oxide synthases, see below), the concentration of nitric oxide in the upper intestine is up to 10,000 times higher (McKnight *et al.*, 1997).

A low pH in the fasting stomach (pH 1-2) is considered too low for microbial growth and, as a consequence, for bacterial nitrate reduction. However, in normal healthy adults a significant proportion (30-40 %) of the population was found to have a fasting pH over 5, which results in increased bacterial activity and hence nitrite levels (Ruddell *et al.*, 1976; Müller *et al.*, 1984). Infants younger than 3 months are highly susceptible to gastric bacterial nitrate reduction to nitrite because they have very little production of gastric acid (Ellen and Schuller, 1983; Kross *et al.*, 1992). Gastrointestinal infections in infants may produce an additional increase in the reduction of nitrate to nitrite.

Nitrate undergoes active secretion in humans not only in the salivary duct cells but also in the gastric parietal cells and occurs at a number of other sites leading to enterosystemic cycling of nitrate and nitrite. Additionally nitrate biotransformation is complex and involves nitrate reduction, nitrite formation, nitrite reoxidation to nitrate, and resulting methaemoglobin in a dynamic equilibrium (Lundberg *et al.*, 2004 and 2008; Gladwin, *et al.*, 2005). Nitrite appears to have a transient role with nitrate being the normal state.

Endogenous nitrate/nitrite formation

There are many reports of an excess of urinary nitrate excretion compared with that ingested at low nitrate intakes (Bartholomew and Hill, 1984; Lee *et al.*, 1986; Gangolli *et al.*, 1994). Ellen and Schuller (1983) suggested that a part of this excess excretion could originate from the inhalation of nitrate and nitrite from indoor and outdoor air and cigarette smoke, although the main part most probably originates from endogenous synthesis.

The main source of endogenous nitrate in mammals is the L-arginine-NO synthase pathway, which is constitutively active in numerous cell types throughout the body. Nitric oxide is produced from the amino acid L-arginine and molecular oxygen by nitric oxide synthetase (NOS). Under basal conditions, the metabolites of endogenous nitric oxide in plasma are mainly derived from the L-arginine-NO pathway in the endothelium of blood vessels and possibly neuronal tissue. However, during systemic inflammatory reactions or infections, white blood cells express an inducible NOS, which produces large amounts of nitric oxide and ultimately, by the binding to oxidised haemoglobin, results in methaemoglobin and a considerable increase in the concentrations of nitrate in plasma (Lundberg *et al.*, 2004 and 2008). In tissues other than the blood, nitrite is formed by reductive pathways and further oxidation produces nitrate (Jensen,

2005; Gladwin, *et al.,* 2005). For example, in the dog large quantities of nitrate were excreted in the bile when the dogs received ^{15}N-labelled nitrite, indicating endogenous oxidation of nitrite (Fritsch *et al.*, 1985).

In recent years, the function of nitric oxide in vascular physiology has become better understood and nitrite is now considered to be a nitric oxide donor under physiological conditions. Thus, low oxygen pressure, low pH and high nitrite concentration favour nitric oxide formation from nitrite, and in mammalian red blood cells nitrite is thus reduced to nitric oxide by deoxyhaemoglobin (Cosby *et al.*, 2003; Gladwin *et al.*, 2004). On the other hand, oxidized haemoglobin will react with nitrite to form nitrate and methaemoglobin (Kosaka and Tyuma, 1987). Normal levels of methaemoglobin in human blood are 1-3 %, and reduced oxygen transport has been noted clinically when methaemoglobin concentration reaches 10 % or more (Walker, 1990; FAO/WHO, 2003a,b). The balance between the two different haemoglobin reactions produces nitric oxide at low oxygen pressure and the vasodilation induced by nitric oxide will increase blood flow to reverse the situation (Jensen, 2005).

Nitrosamine formation

In healthy human volunteers, N-nitrosomorpholine was detected in stomach samples, and the level increased after ingestion of nitrate. Radioactive labelled nitrogen confirmed that the nitrosamine-nitrogen originated from nitrate, demonstrating *in situ* formation of N-nitrosamine from dietary nitrate via nitric oxide (Winter *et al.*, 2007). Nitrosamines were formed in the gastrointestinal tract of Sprague Dawley rats after feeding a normal rat chow, and also in rats fed semipurified diets mixed with meat or hot dogs. In the latter case, the nitrosamine levels increased 2-3 times above control (semipurified diet alone). In a dynamic *in vitro* gastrointestinal model, the formation of N-nitrosodimethylamine (NDMA) was observed after gradually adding nitrite to food samples (fish). For some of the samples the model produced measurable NDMA levels, and the addition of orange juice or tea (antioxidants) generally decreased the NDMA formation (Krul *et al.*, 2004).

Thus overall, when nitrate is consumed as part of a normal diet containing vegetables, other bioactive substances concomitantly consumed such as the antioxidant vitamin C can reduce the amount of nitrosamine formed by up to half (Brambilla and Martelli, 2007).

Excretion

About 25 % of an oral nitrate dose was secreted in the saliva (Eisenbrand *et al.*, 1980; Speijers *et al.*, 1987; Kortboyer *et al.*, 1995; Lundberg *et al.*, 1994; FAO/WHO, 2003a), but there were marked inter-individual and diurnal variations in this secretion (Bartholomew and Hill, 1984; Cortas and Wakid, 1991). In a study on healthy volunteers that were administered an oral dose of 10 mg sodium nitrate/kg b.w. and monitored for one day, the cumulative salivary nitrate excretion, expressed as percentage of the ingested dose, was 28 % (Kortboyer *et al.*, 1995). In the minipig, an appropriate model for humans in terms of salivary secretion, bilateral removal of the

parotid glands led to a significant decrease of nitrate secretion from blood to saliva, and thus low nitrite levels. The study suggests that the parotid salivary glands play an important role in the balance of nitrate and nitrite levels in the body (Xia *et al.*, 2003).

Single oral gavage of varying doses of potassium nitrate gave a urinary nitrate excretion of 65-70 % irrespective of dose. Excretion was maximal 5 h after ingestion and returned within 18 h to baseline levels, which in fasting subjects were 10-20 mg/litre (Bartholomew and Hill, 1984; Tannenbaum and Green, 1981; Wagner *et al.*, 1983). Results indicate a predominantly tubular excretion of nitrate (Ellen at el., 1982). In a study on healthy infants, the urinary excretion of nitrate (average 8.7 mg nitrate/day) was as high as, or even higher, than a low (average) intake of 2-7 mg nitrate plus nitrite per day. The authors concluded that excretion probably included endogenously formed nitrate (Hegesh and Shiloah, 1982). In the anaesthetized dog, urinary excretion rates of nitrate increase progressively in response to increases in the circulating levels without exhibiting a maximum; however, there was a progressive decrease in fractional reabsorption with increasing dose (Godfrey and Majid, 1998). It should be noted that a major part of the primary urinary nitrate (ca 80%) is pumped back to the blood by an active transport mechanism (Kahn *et al.*, 1975). This salvaging of nitrate from the urine, in addition to the known recycling of nitrate from saliva and also from the intestines (after biliary excretion) strongly suggests that the body is acting to conserve a substance of physiological importance.

In faeces, low levels of nitrate and nitrite are present (Saul *et al.*, 1981; Wagner *et al.*, 1983). However, the observed conversion of nitrate to nitrite by the faecal microflora suggests that biliary excretion of nitrate may be higher than the amount detected in the faeces (Archer *et al.*, 1982; Saul *et al.*, 1981). In a model developed by Schultz and co-workers (1985) the bacteria of the large intestine were suggested to be responsible for about half of the extrarenal removal of nitrate from the body.

Levels up to 5 mg nitrate/kg breast milk have been reported (Sugekawa and Matsumoto, 1975). Nitrate levels in milk from lactating women after a normal meal did not exceed the simultaneously measured maternal plasma nitrate levels (Green *et al.*, 1982).

Summary

In conclusion, once nitrate is ingested it is quickly absorbed from the gastrointestinal tract into the plasma in humans. About 25% of the plasma nitrate is taken up by the salivary glands, bioconcentrated approximately 10-fold and secreted into the saliva. In the mouth, bacterial reduction of approximately 20% of the secreted nitrate to nitrite occurs, normally constituting 5-7% of the absorbed nitrate dose in healthy adults. In the stomach, under acidic conditions, nitrite will be transformed to nitric oxide and other metabolites. Most of the absorbed nitrate is ultimately excreted in the urine, but considerable salvage takes place in advance through selective reabsorption from the kidney together with biliary and salivary recirculation.

8.2 General toxicology

The toxicity of nitrate is known to be low and adverse effects have been shown to arise from its metabolic conversion to nitrite (EC, 1997). This section presents a short summary of the toxicology of nitrate and nitrite with particular emphasis on the potential for human health effects. A full account of all toxicological studies will not be provided since this has been carried out previously by the JECFA (FAO/WHO, 2003a,b) and no new significant data have been found.

8.2.1 Acute toxicity

8.2.1.1 Nitrate

The acute oral toxicity of nitrate in animals is generally low with LD_{50} values of approximately 2500-6250 mg/kg b.w./day in mice, 3300-9000 mg/kg b.w./day in rats, 1900-2680 mg/kg b.w. in rabbits and 300 mg/kg b.w. in pigs (Walker, 1990, Speijers, *et al.*, 1987). It has been observed that the oral lethal dose of nitrate in humans is around 330 mg/kg b.w (Walker, 1990).

8.2.1.2 Nitrite

Sodium nitrite is approximately 10-fold more toxic than sodium nitrate depending on the species with LD_{50} values of 214 mg/kg b.w. in mice, 180 mg/kg b.w. in rats and 186 mg/kg b.w. in rabbits (NIOSH, 1987).

8.2.2 Sub-chronic toxicity

8.2.2.1 Nitrate

No new subchronic studies have been identified for nitrate. Historically, no adverse effects were observed in two dogs after dosing sodium nitrate in the diet at a level of 2% for 105 and 125 days (Lehman, 1958) calculated to be equivalent to 500 mg/kg b.w./day corresponding to 370 mg/kg b.w. nitrate (Walker, 1990). Short term studies in rats dosed up to 10% sodium nitrate in drinking water over 6 weeks showed slight elevation of methaemoglobin.

8.2.2.2 Nitrite

A 14 week study was conducted in B6C3F1 mice (10 males and 10 females/group) with dose levels of 0, 375, 750, 1500, 3000, or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 90, 190, 345, 750, or 990 mg sodium nitrite/kg b.w. to males and 120, 240, 445, 840, or 1230 mg sodium nitrite/kg b.w. to females) in drinking water. Overall at the highest dose, body weight, spleen weight and sperm counts were lower in males compared to controls and in females, absolute and relative organ weights (heart, kidney, liver and spleen), together with the length of estrous cycle, were impaired. Histopathological examination showed that squamous cell hyperplasia of the forestomach and extramedullary haematopoiesis were more frequent at the two highest dose levels in both sexes. Degeneration of the testis was seen in

males at 750 mg/kg b.w. and above. The NOAEL was concluded to be 190 mg/kg b.w./day (NTP, 2001).

A 14 week study was conducted in male and female rats (10 males and 10 females/group) at dose levels of 0, 375, 750, 1500, 3,000, or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 30, 55, 115, 200, or 310 mg sodium nitrite/kg b.w. to males and 40, 80, 130, 225, or 345 mg sodium nitrite/kg b.w. to females) in drinking water. Elevated methaemoglobin (metHb) was observed at all dose levels. Sperm motility was the endpoint related to a no observed effect level (NOEL) of 55 mg/kg for sodium nitrite corresponding to 37 mg/kg for nitrite (NTP, 2001).

Methaemoglobin results from the reaction of nitric oxide with oxyhaemoglobin at the same time forming nitrate. A number of factors are critical to metHb formation including the presence of increased nitrite, intestinal infection together with inflammation of the stomach lining and NADH-cytochrome b5 methaemoglobin reductase (which converts methaemoglobin back to haemoglobin). Methaemoglobin is produced normally with background levels of 1-3%. Levels of 10% or more have been shown clinically to reduce oxygen transport. At levels above 20%, cyanosis and hypoxia can occur and an increase to 50% methaemoglobin can prove fatal (Mensinga *et al.*, 2003). Infants younger than 3 months of age are more susceptible to methaemoglobinaemia than adults due to a 40-50 % lower activity of NADH-cytochrome b5 methaemoglobin reductase r (which converts methaemoglobin back to haemoglobin) and their increased risk for intestinal infections (Savino *et al.,* 2006).

Hypertrophy of the adrenal zona glomerulosa has been investigated in a 13-week study using Wistar rats. A no observed adverse effect level (NOAEL) of 5.4 mg/kg b.w./day for the nitrite was found (Til *et al.,* 1997). The mechanism is considered to involve nitrite-induced vasodilatation via nitric oxide production and a reduction in blood pressure activating the renin-angiotensin system in the kidney. Consequential mechanisms to restore the physiological blood pressure result in the production of the vasoconstrictor angiotensin II and release of aldosterone from the adrenal zona glomerulosa, resulting in hypertrophy of the zona glomerulosa (Boink *et al.,* 1998; Mensinga *et al.,* 2003).

8.3 Genotoxicity

From the peer-reviewed literature, sodium nitrate was not found to be mutagenic in *in vitro* tests. For nitrite, *in vitro* mutagenic potential was shown in *Salmonella typhimurium* strain TA100 both with and without metabolic activation but not in strain TA98 (NTP, 2001).

In vitro culture of peripheral blood lymphocytes has been used to evaluate the ability of nitrate and nitrite to produce chromosome aberrations in mammalian cells. Sodium nitrate did not increase aberrations (17.6-70.6 mM) but high doses of sodium nitrite (14.4 mM) resulted in a slight increase in micronucleated cells and chromatid gaps (Balimandawa *et al.*, 1993). However, *in vivo* no micronuclei induction occurred in the bone marrow of rats and mice after intraperitoneal injection and a test for micronuclei in peripheral blood from mice in the 14-week

study (described above) also gave negative results suggesting that overall sodium nitrite is not genotoxic *in vivo* (NTP, 2001, FAO/WHO, 2003a,b).

A significant increase in the mean number of chromatid/chromosome breaks was reported in a group of Greek children exposed to nitrate concentrations above 70.5 mg nitrate/L in drinking water compared to a control group exposed to very low nitrate concentrations (i.e., 0.7 mg/L).There was no significant increase in the mean number of sister chromatid exchanges per cell (Tsezou *et al.*, 1996).

Overall, the JECFA concluded that there was no evidence for the reclassification of either nitrate or nitrite as genotoxic compounds (WHO/FAO, 2003a,b).

8.4 Chronic toxicity/ carcinogenicity

8.4.1 Nitrate

A number of long-term toxicity/carcinogenicity studies have been performed. Firstly, rats were given 0, 0.1, 1, 5 and 10 % sodium nitrate in the diet for 2 years (Lehman, 1958; Walker, 1990) equivalent to 0, 50, 500, 2,500 and 5,000 mg/kg b.w./day (Walker, 1990). A NOEL of 500 mg/kg b.w./day was established for sodium nitrate based on a slight depression in growth rate and inanition at higher doses. No adverse histological changes or increase in tumour frequency were found (Lehman, 1958 as cited by WHO/FAO, 1962). Secondly, rats were dosed with 0 or 0.5% sodium nitrate in drinking water over 84 weeks (Lijinsky *et al.*, 1973) calculated to be equivalent to 0 and 500 mg/kg b.w./day (Walker, 1990). No histopathological effects of treatment were observed. Thirdly, in a more recent 2 year study rats were given 0, 2.5 and 5 % sodium nitrate in drinking water (Maekawa *et al.*, 1982) calculated to be equivalent to 0, 2,500 and 5,000 mg/kg b.w./day (Walker, 1990). At 5,000 mg/kg b.w./day, slight to moderate reduced body weight gain was observed. From this study a NOAEL of 2,500 mg/kg/b.w/day for nitrate was derived. Overall, these studies demonstrate a low chronic toxicity of nitrate.

8.4.2 Nitrite

In a two year chronic toxicity study in rats given nitrite in the drinking water equivalent to doses of 0, 10, 100, 200 and 300 mg/kg b.w./day no significant differences between control and treated groups were shown for growth, mortality and total haemoglobin levels. At the highest three doses, methaemoglobin increased to 5, 12 and 22% and lung toxicity was observed with dilatation of the bronchi with infiltration of lymphocytes and emphysema. At the highest dose, focal degeneration and fibrosis of the heart muscle as well as dilatation of coronary arteries were also observed. Based on heart and lung toxicity the NOAEL for sodium nitrite was 10 mg/kg b.w./day and hence the NOAEL for the nitrite ion was 6.7 mg/kg b.w./day (Gruener and Shuval, 1973).

More recently, two year carcinogenicity studies for sodium nitrite were conducted under the National Toxicology Programme (NTP, 2001) in B6C3F1 mice and F344/N rats. In the mouse study, 50 male and 50 female B6C3F1 mice were exposed through drinking water to daily doses

equivalent to 0, 60, 120, or 220 mg/kg b.w./day and 0, 45, 90, or 165 mg/kg b.w./day respectively. Overall, there was no difference in survival between exposed groups compared to controls although mean body weights were lower in females treated with the highest dose. Exposed groups generally consumed less water than the control groups. The incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female mice occurred with a "positive dose-related trend" (not statistically significant) with respective frequencies of 1/50, 0/50,1/50 and 5/50 at 0, 45, 90, or 165 mg/kg b.w./day respectively. The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in males treated at the highest dose. In females, the authors concluded that there was equivocal evidence[16] for carcinogenic activity (NTP, 2001) based on the trend in the combined incidence of squamous cell papilloma and carcinoma of the forestomach.

8.5 Endocrine toxicity

Nitrate intake could have the potential to adversely affect thyroid function as nitrate shares the same transport mechanism as iodide. This inhibition could lead to a decrease in circulating thyroid hormone levels with feedback resulting in compensatory thyroid gland enlargement (goitre). To investigate this a four week oral study performed in human volunteers showed that sodium nitrate exposure of three times the ADI (15 mg/kg b.w./day in water) did not cause changes in the thyroid gland function (Lambers *et al.*, 2000).

8.6 Derivation of the acceptable daily intakes for nitrate and nitrite

The former SCF and the JECFA both derived ADIs for nitrate and nitrite. The SCF reviewed the toxicological effects of nitrate and nitrite and established an ADI of 0-3.7 mg/kg b.w. for nitrate in 1990 (EC, 1992), retained the ADI in 1995 and derived an ADI of 0-0.06 mg/kg for nitrite (EC, 1997). The most recent assessment of nitrate and nitrite in 2002 by the JECFA reconfirmed the ADI of 0-3.7 mg/kg b.w. for nitrate and set an ADI of 0-0.07 mg/kg b.w. for nitrite based on a long term NTP rat study (FAO/WHO, 2003a,b). In the absence of significant new toxicological and toxicokinetic data, the Panel concluded that there was no need to re-consider these ADIs.

The key studies used to derive the ADIs are summarized in Table 18. A NOEL of 500 mg/kg b.w./day sodium nitrate corresponding to 370 mg/kg b.w. nitrate was derived from long term studies in rats and the subchronic toxicity study in dogs. Applying an uncertainty factor of 100 resulted in ADIs of 0-5 and 0-3.7 mg/kg b.w./day for sodium nitrate and nitrate, respectively. It has been argued that the rat may not be a good model for humans due to its low conversion of nitrate into nitrite in the saliva. However, because of the importance of the chronic toxicology, the rodent toxicokinetics and similar NOAELs found in the dog (a relevant model for humans) these studies continue to be considered to be relevant for risk assessment.

[16] The term equivocal evidence of carcinogenic activity is defined in NTP as studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.

The JECFA (FAO/WHO, 1995) also considered the conversion of nitrate to nitrite in the saliva in its assessment using the calculation developed by the SCF (EC, 1997) to derive a transposed NOAEL for nitrate based on the NOAEL for nitrite. These "transposed" NOAELs can be compared with the current ADI of nitrate. The JECFA (FAO/WHO, 1995) applied an uncertainty factor of 50 to the "transposed" NOAEL for normal converters of 160 mg/kg b.w day which resulted in an ADI of 3.2 mg/kg b.w day. Because this was in the same range as the ADI for nitrate (3.7 mg/kg per day), there was no justification to amend this ADI in the JECFA 2002 assessment (FAO/WHO, 2003a).

Table 18. Summary of the NOELs from toxicological studies used to derive ADI values for nitrate and nitrite in the latest the JECFA (FAO/WHO, 2003a,b) evaluation.

Type of study	Toxicological Endpoint	NOEL[17] sodium salt/anion mg/kg b.w./day	ADI sodium salt/anion mg/kg b.w./day	Reference
Nitrate				
Subchronic study in dogs (125 days)	Growth depression	500/370	5.0/3.7	Lehman, 1958 cited in JECFA 1962
2 year chronic study in rats	Growth depression	500/370	5.0/3.7	Lehman, 1958 cited in JECFA 1962; Lijinski, 1973
Nitrite				
2 years study in rats	Heart and lung toxicity	10/6.7	0.1/0.07	Maekawa *et al.*, 1982

[a] mg/kg body weight per day

8.7 Human data

8.7.1 Introduction on epidemiological studies

Epidemiological study designs can be ranked according to increasing strength of evidence: ecologic (or correlation) studies, cross-sectional, case-control, cohort studies, intervention trials. A classification of the different epidemiologic study designs with respect to their potential for bias to occur and, consequently, the strength of evidence they provide, and the costs involved has been described (van den Brandt *et al.*, 2002). It indicates that intervention trials provide the strongest evidence for a causal relationship on risk and (due to the ability of the design to control for confounding and bias), have the lowest chance for potential bias to occur. However, they are the most expensive and usually the least feasible studies. The less expensive cohort studies assess exposure and select study participants before the health outcome of interest occurs and thus provide relatively strong evidence. Although the cheaper case-control studies generally assess

[17] The term NOEL has been used by the JECFA until 2007 See URL: http://www.fao.org/ag/agn/agns/files/jecfa68_final.pdf

exposures retrospectively in subjects with and without the health outcome, the resultant evidence is more debatable. This is particularly true in the case of dietary exposures, due to the possibility of selection bias, recall bias and/or presence-of-disease bias to occur. The lowest costs are associated with correlation (ecological) studies but, as mentioned previously, they provide weak evidence and are much more susceptible for bias. Some investigators have stated that observational studies cannot, by definition, establish causality of a relationship based on a statistical association. However, if several high quality studies, such as those in which biases are shown to be minimal are available, and these consistently show a dose-response association, then observational studies may very well contribute to conclusions about causality.

Because vegetable consumption may confer some degree of protection against cancer, negative confounding may be present in studies linking nitrate exposure from vegetables to a cancer risk.

8.7.2 Relationship between nitrate (and nitrite) intake and possible health effects in humans

The JECFA report 2003

The relationship between nitrate and nitrite intake and human health has been considered in earlier reviews (e.g., Gangolli *et al.*, 1994) and by the JECFA at its 59th meeting, which included literature until 2002 (FAO/WHO, 2003a,b). The part of the JECFA report on nitrate intake, methaemoglobinaemia risk and human cancer risk can be summarized as follows.

The results of studies in humans on the potential of a high nitrate intake to cause methaemoglobinaemia were equivocal. Some of the studies showed an association between a high nitrate concentration in drinking-water and methaemoglobinaemia, and others indicated that gastrointestinal infections, inflammation and the ensuing overproduction of nitric oxide are major factors in infantile methaemoglobinaemia. No increase in methaemoglobin concentration was seen in volunteers after a single administration of sodium nitrate in drinking water providing a dose of 7.3 mg/kg b.w., expressed as nitrate.

Several studies were reviewed on the effect of administration of nitrate on the release of nitric oxide at the junction of the oesophagus and the stomach in humans, which, it had been speculated, might be associated with an increased incidence of cancer at this site. However, no such association has been observed in epidemiological studies.

Six ecological (correlation) studies were reported on nitrate in drinking-water and mortality from or incidence of cancer. Elevated risks were found for prostate cancer and for brain tumours (each in one study), but the results of six studies on gastric cancer were equivocal.. Furthermore, most of the ecological studies were based on limited data on nitrate concentrations and on cancer mortality rates (rather than incidence rates), and none took an induction period for cancer into account.

Three of the studies were cross-sectional, involving measurement of, e.g., salivary nitrate in cancer patients and healthy subjects. Because cross-sectional studies do not take into account the

time between exposure and disease, any observed differences in biomarkers of exposure might also be a consequence of the disease; therefore these studies cannot contribute to a causal interpretation of the results of studies of nitrate intake and cancer risk.

Seven case–control studies on nitrate in drinking-water and/or food and cancers at various sites were reviewed. In the studies on nitrate in drinking-water, equivocal results were reported with regard to an association with non-Hodgkin lymphoma, and no association was found with brain tumours. In the studies on dietary nitrate, no association was found with oral, oesophageal, gastric or testicular cancer. No other cancer sites have been studied.

Three prospective cohort studies have been conducted on nitrate intake and cancer risk. A cohort study in the Netherlands, with 6 years of follow-up, found no significant association between the incidence of gastric cancer and intake of nitrate from food or drinking-water, with relative risks for increasing quintiles of total nitrate intake of 1.0 (reference quintile), 1.2, 0.7, 0.9 and 0.9 for mean intakes of 60, 85, 100, 120 and 180 mg/day, respectively. Neither the relative risks nor the trend across relative risks was significant. A further analysis of the effect of nitrate within tertiles of vitamin C intake also did not reveal a positive association between nitrate intake and gastric cancer (van Loon *et al.*, 1998). A Finnish cohort study on dietary nitrate, with 24 years of follow-up, reported no association with the risks for tumours of the stomach, colorectum or head and neck. The average nitrate intake in this cohort was reported to be 77 mg/day (Knekt *et al.*, 1999). A cohort study in Iowa, USA, with 11 years of follow-up, revealed no consistent association between intake of nitrate from drinking-water and the risks for cancers at many sites, and an inverse association was reported with cancers of the uterus and rectum. Positive associations with nitrate intake were observed only for cancers of the ovary and urinary bladder, although it was not possible to determine whether other factors in drinking-water were responsible for these associations. In addition, no evidence of a dose–response relationship was found for any of the cancer sites addressed in the study in Iowa (Weyer *et al.*, 2001). The cohort studies included control for various potential confounders, such as intake of vegetables, age and smoking.

Overall, the epidemiological studies reviewed by the JECFA at its 59th and 44th meeting, did not provide evidence that nitrate is carcinogenic to humans (FAO/WHO, 2003a,b).

The part of the JECFA report on nitrite intake and human cancer risk regarding literature until 2003 can be summarized as follows. A number of epidemiological studies of the relationship between the intake of nitrite and cancer risk had been published since the 44th meeting. The JECFA ranked the study designs according to their capacity to provide evidence of a relationship.

Nine case–control studies on previous nitrite intake and various cancer types were reviewed. For oral and laryngeal cancer, no association was found with nitrite intake. One study conducted in the USA reported a positive association with oesophageal cancer, with Odds Ratio (ORs) of 1.0 (reference category), 1.2 and 1.6 for persons with a daily nitrite intake of < 1.1 mg, 1.1–1.6 mg and > 1.6 mg, respectively. The ORs and the trend across ORs were not statistically significant, however. The association between nitrite intake and oesophageal cancer was stronger, and it was significant for persons with a history of cancer (as an indicator of possible endogenous nitrosation) (Rogers *et al.*, 1995). Another study in the USA, however, found no association

between nitrite intake and oesophageal cancer, nor with the subtypes adenocarcinoma and squamous-cell carcinoma; a positive association was found only with gastric cancer other than of the cardia (Mayne *et al.*, 2001). A positive association with gastric cancer was reported in an Italian case–control study (average consumption, 2.4 mg/day) (La Vecchia *et al.*, 1997), while no association was found in a French study (average consumption, 1.9 mg/day) (Pobel *et al.*, 1995).

An association of borderline significance was found between nitrite intake and urinary bladder cancer in men but not women of Japanese descent, nor in whites of either sex, in Hawaii, USA (Wilkens *et al.*, 1996). Although a positive association was reported from a study in the USA between brain tumours in children and their mothers' consumption of processed meat (Preston-Martin *et al.*, 1996), no association was found with nitrite intake during gestation or in childhood in a recent case–control study from Israel (Lubin *et al.*, 2000). One study on nasopharyngeal cancer among Taiwanese reported no association with nitrite intake in adulthood, but a positive association was found with childhood nitrite intake as recalled by the mothers of the cases and controls (Ward *et al.*, 2000).

Two prospective cohort studies have been conducted on nitrite intake and cancer risk. A cohort study from the Netherlands, with 6 years of follow-up, on dietary nitrite and gastric cancer risk reported relative risks of 1.0 (reference category), 1.2, 1.2, 0.9 and 1.4 for increasing mean quintiles of nitrite intake of 0.01, 0.04, 0.09, 0.16 and 0.35 mg/day, respectively. Neither the relative risks nor the trend was significant (van Loon, *et al.*, 1998). A Finnish cohort study, with 24 years of follow-up, reported no association with the incidence of stomach, colorectal, or head-and-neck tumours. The average nitrite intake by this cohort was reported to be 5.3 mg/day (Knekt *et al.*, 1999).

Thus, some studies indicated increased risks for oesophageal and gastric cancer; however, other studies – particularly prospective cohort studies – revealed no such association. The results for brain tumours in children and for urinary bladder cancer in adults were equivocal. Wide variation between the studied populations in the recorded intake of nitrite was noted. In none of these studies was a possible interaction between nitrite and nitrosatable amines evaluated in respect of cancer risk.

The results of these studies and those of the epidemiological studies considered by the JECFA at its 44th meeting did not provide evidence that nitrite is carcinogenic to humans-(FAO/WHO, 2003a,b).

New studies since the JECFA 2003 report

In the following, new epidemiological studies that have been published from 2002 onwards will be summarized, categorized according to study design and strength of evidence.

Nitrate (and nitrite) and methaemoglobinaemia

In a case-control study nested in a cohort, risk factors for methaemoglobinaemia in 71 children were investigated in the Transylvania region of Romania, where wells are a very important water source. Univariate and multifactorial analysis of risk factors for methaemoglobinaemia emphasised that, for this population, methaemoglobinaemia is most strongly associated with nitrate/nitrite exposure through the dietary route ($p = 0.0318$), via feeding of infant formula and tea made with water containing high levels of nitrate (253 mg/L in the exposed group versus 28 mg/L in the control group). Moreover, breast-feeding was found to be protective in infants younger than 6 months of age ($p = 0.0244$). Mean reported nitrate intake levels among case and controls were 103.6 and 11.2 mg/kg/b.w/day, respectively. The findings also raise questions about the role of diarrhoeal disease in the development of methaemoglobinaemia, as multifactorial analysis indicated a significant role for diarrhoeal disease for some individuals (Zeman *et al.*, 2002).

Relationship between chronic nitrate (and nitrite) intake and possible risk of cancer in humans

Ecologic studies

An ecologic study on nitrate levels in drinking water and non-Hodgkin lymphoma (NHL) and cancers of the digestive and urinary tracts was conducted in an agricultural district (Trnava District; population 237,000) of the Slovak Republic. Routinely collected nitrate data (1975-1995) for villages using public water supplies were computerized and linked to cancer incidence ascertained for the period 1986-1995. Increasing standardized incidence ratios (SIRs) for villages with low average levels of total nitrate in drinking water (0-10 mg/L), medium (10.1-20 mg/L), or high (20.1-50 mg/L) were seen for colorectal cancer in women (0.64, 1.11, 1.29; P for trend <0.001) and men (0.77, 0.99, 1.07; P for trend=0.051), and non-Hodgkin lymphoma in women (0.45, 0.90, 1.35; P for trend=0.13) and men (0.25, 1.66, and 1.09; P for trend=0.017). There were no associations for kidney or bladder cancer. These ecologic data support the hypothesis that there is a positive association between nitrate in drinking water and NHL and colorectal cancer (Gulis *et al.*, 2002).

In an ecologic study, Cocco and co-workers compared the NHL incidence in 1974-1993 with nitrate monitoring data from community water supplies from 1971-1994 available for 75% of the 376 communes in Sardinia, Italy. Among the study communes, the average nitrate concentration in 2003 was 4.57 mg/L (SE 0.35; median 3.27). The relative risks (RRs) for NHL for men and women combined did not increase with increasing 1993 nitrate level. Among men, the RRs were significantly increased in some nitrate concentration categories. Among women, the RRs were not increased in any exposure category. There was limited evidence among men for an association with NHL, but not among women (Cocco *et al.*, 2003).

Cross-sectional studies

No new studies were reported since 2002.

Case-control studies

Stomach cancer

A case-control study was conducted in Korea to assess gastric cancer (GC) risk in relation to dietary intake of nitrate. Trained dieticians interviewed 136 patients diagnosed with GC, and the same number of controls was selected by matching sex, age and hospital. Intake of citrus fruits rather than total fruits was shown to have a protective effect on the risk of GC, but was not significant. Intake of citrus fruits rather than total fruits was shown to have a protective effect on the risk of GC with ORs of 0.60 and 0.66 in medium and high consumers (95% confidence interval [CI] = 0.33-1.10 and 0.31-1.41, but was not significant (P for trend=0.27). In this study, intake of total vegetables was shown to have a protective effect with OR for GC of 0.43 and 0.64 in medium and high consumers respectively (95% confidence interval [CI] = 0.23-0.80 and 0.31-1.32, P for trend=0.025). However, an increased risk for GC was shown in medium (OR=1.67, [CI] =0.87-3.2) and high consumers (OR=2.17 [CI] =1.02-4.65) of high nitrate-containing vegetables but it did not reach statistical significance (P for trend=0.18) (Kim *et al.*, 2002).

Other gastrointestinal cancers

The association of nitrate in public water supplies with incidence of colon and rectum cancers was studied in a case-control study conducted in Iowa, USA, from 1986 to 1989. Nitrate levels in Iowa towns were linked to the participants' water source histories. Analyses were focused on the period from 1960 onward, during which time nitrate measurements were more frequent, and analyses were restricted to those persons with public water supplies that had nitrate data (actual or imputed) for greater than 70% of this time period (376 colon cancer cases, 338 rectum cancer cases, and 1244 controls). There were no overall associations of colon or rectum cancers with measures of nitrate in public water supplies, including average nitrate and the number of years with elevated average nitrate levels. For more than 10 years with average nitrate greater than 5 mg/L, the OR for colon cancer was 1.2 ([CI] = 0.9-1.6) and for rectum the OR was 1.1 (CI = 0.7-1.5). However, nitrate exposure (>10 years with average nitrate >5 mg/L) was associated with increased colon cancer risk among subgroups with low vitamin C intake (OR = 2.0; CI = 1.2-3.3) and high meat intake (OR = 2.2; CI = 1.4-3.6). These patterns were not observed for rectum cancer. (De Roos *et al.*, 2003).

Non-Hodgkin Lymphoma (NHL)

A population-based case-control study of NHL was conducted in 1998 to 2000 in Iowa, Detroit, Seattle, and Los Angeles. Monitoring data for public supplies were linked to water source histories from 1960 onward. Nitrate was measured at interview homes with private wells. For those in the diet arm, dietary nitrate and nitrite intake were estimated using a 117-item food-frequency questionnaire that included foods high in nitrate and nitrite. In multivariate analyses,

no overall association was found with the highest quartile of average drinking water nitrate (> 2.90 mg/L nitrate-N: odds ratios = 1.2; 95% confidence interval = 0.6-2.2) or with years > or = 5 mg/L (10+ years: 1.4; 0.7-2.9). No evidence of an interaction was seen between drinking water nitrate exposure and either vitamin C or red meat intake, an inhibitor and precursor, respectively, of N-nitroso compound formation. Among those in the diet arm, dietary nitrate was inversely associated with risk of NHL (highest quartile: 0.54; 0.34-0.86). Dietary nitrite intake was associated with increasing risk (highest quartile: 3.1; 1.7-5.5) largely due to intakes of bread and cereal sources of nitrite. Average drinking water nitrate levels below 3 mg/L were not associated with NHL risk (Ward *et al.*, 2006).

Brain tumours in adults

A population-based case-control study of adult glioma in eastern Nebraska, USA, was carried out with 236 glioma cases and 449 controls using information obtained from a food-frequency questionnaire. After adjusting for potential confounders, inverse associations with risk of adult glioma were observed for intakes of dark yellow vegetables (highest quartile versus lowest: OR = 0.6, P trend = 0.03) and beans (OR = 0.4, P trend = 0.0003), but no associations were seen for dietary sources of preformed nitrosamines or high-nitrate vegetables. No significant associations were observed with risk of adult glioma for intakes of nitrate, nitrite, vitamin C, vitamin E, saturated fat, cholesterol, dietary fibre from grain products, or fibre from vegetables and fruit. The authors concluded that this study did not support the N-nitroso compound hypothesis for adult glioma (Chen *et al.*, 2002).

In a further extension of this case-control study of adult glioma in Nebraska, drinking water nitrate and nitrite were also considered. Water utility nitrate measurements were linked to residential water source histories. Average nitrate exposure over a 20-year period was computed. A food frequency questionnaire was used to assess dietary nitrate and nitrite. Increasing quartiles of the average nitrate level in drinking water were not significantly associated with risk (adjusted odd ratios: 1.4, 1.2, 1.3). Risk was similar among those with both higher and lower intakes of vitamin C. Dietary nitrite intake was not associated with risk. The authors concluded that this study did not support a role for drinking water and dietary sources of nitrate and nitrite in risk of adult glioma (Ward *et al.*, 2005).

Childhood brain tumours

Pogoda and Preston-Martin (2001), building on the earlier case-control study with 540 cases and 801 controls from the USA by Preston-Martin *et al.*, (1996), reported a further analysis with refined nitrite intake calculations. They found a positive association between childhood brain tumours (CBT) and their mothers' intake of nitrite from cured meat, which was only significant in the highest nitrite intake category: they observed a 2-3 fold increased risk in the offspring of mothers who consumed on average at least 3 mg nitrite from cured meat per day during pregnancy. Distinction between total nitrite intake and cured meat was not available from the study and no further subdivision of CBT was made in the analysis.

Mueller *et al.*, (2004) conducted a multicentre case-control study in France, Italy, Spain, Canada and the USA on drinking water levels of nitrate and nitrite and risk of CBT, with 836 CBT cases and 1485 controls. They found no increased CBT risk with increasing nitrate from drinking water. However, the risk of astrocytoma was significantly positively associated with increasing nitrite levels in residential drinking water during pregnancy: the odds ratios (and 95% CI) were 4.3 (1.4 – 12.6) for nitrite levels of 1 - <5 mg/L nitrite and 5.7 (1.2 – 27.2) for nitrite levels ≥5 mg/L. There was no association with other CBT.

Other cancers

A population-based case-control study of bladder cancer (men and women) and nitrate in drinking water was conducted in Iowa, USA, using 808 cases and 1259 controls. Among controls, the median average nitrate level for their Iowa residences with public water supplies was 1.3 mg/litre nitrate-nitrogen (interquartile range = 0.6-3.0). After adjustment for confounders, no increased risk of bladder cancer was found with increasing average nitrate levels in drinking water; the highest quartile odds ratio for women was 0.8 (95% confidence interval = 0.4-0.8), and for men 0.5 (0.4-0.8). In addition, no association was observed among those with high water nitrate exposure (>median) and low (<median) vitamin C intake compared with those who had low water nitrate and high vitamin C intake. These data suggested according to the authors that long-term exposure to nitrate in drinking water at levels in this study (90th percentile 5.5 mg/litre nitrate-nitrogen) is not associated with risk of bladder cancer. Moreover, no increased risk in bladder cancer was concluded when taking into account dietary nitrate levels (> 119 mg/day) and dietary nitrite levels (>1.4 nitrite mg/day) in men and women (Ward *et al.*, 2003).

Cohort studies

The association between nitrate exposure from diet and drinking water and bladder cancer risk was investigated in The Netherlands Cohort Study, conducted among 120,852 men and women, 55-69 years of age at entry. Information on nitrate from diet was collected via a food frequency questionnaire in 1986 and a database on nitrate content of foods. Individual nitrate exposures from beverages prepared with tap water were calculated by linking the postal code of individual residence at baseline to water company data. After 9.3 years of follow-up and after excluding subjects with incomplete or inconsistent dietary data, 889 cases and 4,441 subcohort members were available for multivariate analyses. The multivariate RRs for nitrate exposure from food, drinking water, and estimated total nitrate exposure were 1.06 (95% CI, 0.81-1.31), 1.06 (95% CI, 0.82-1.37), and 1.09 (95% CI, 0.84-1.42), respectively, comparing the highest to the lowest quintiles of intake. Dietary intake of vitamins C and E (low/high) and cigarette smoking (never/ever) had no significant impact on these results, i.e. there was no interaction. The authors concluded that this study did not support an association between nitrate exposure and bladder cancer risk (Zeegers *et al.*, 2006)

The association between intake of nitrite and nitrosamines and gastric cancer (GC) and oesophageal cancer (OC) was evaluated in a recent systematic review. All published case-control

and cohort studies analyzing the relationship between nitrosamines and nitrite intake (and related foods) and GC or OC risk were reviewed. There were 11 cohort studies and 50 case-control studies. Evidence from case-control studies supported an association between nitrite and nitrosamine intake and gastric cancer risk, but was insufficient regarding oesophageal cancer risk. Evidence from cohort studies did not support significantly positive associations (Jakszyn *et al.*, 2006).

Conclusions regarding nitrate, nitrite and human cancer risk

Several ecologic, case-control and cohort studies have been published since the JECFA report (FAO/WHO, 2003a,b; Cogliano *et al.*, 2008). For nitrate, some studies suggest a positive association with risk of NHL, gastric and colon cancer. However, these were mostly studies with a weak study design and limited strength of evidence; other case-control studies and cohort studies (which provide stronger evidence) find no increased risk with increasing nitrate intake after multivariate adjustment. It should be borne in mind however, that the measurement of dietary nitrate intake is not without error and could result in an effect being underestimated. In general, misclassification is nondifferential, leading to attenuation of dose-response relationships. This attenuation applies equally to positive and inverse associations that have been reported for nitrate and cancer, which means that both observed inverse and positive associations might in reality be stronger. Some validation studies have been conducted on nitrate intake measurement; these indicate that the questionnaires are able to rank individuals according to intake, and that the possible attenuation is likely to be moderate. Since the observed associations are often very weak or even null, the CONTAM Panel concluded that, when the newly published data are considered together with studies previously summarized in the JECFA report FAO/WHO, 2003a,b), the evidence does not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk.

For nitrite in food and drinking water, two case-control studies have found that high maternal intakes of nitrite from cured meat or drinking water might be associated with risk of childhood brain tumours. No further cohort studies have been reported on nitrite since the JECFA 2003 report. Taken together, more evidence is available now that a high nitrite intake might be associated with risk of childhood brain tumours and possibly gastric and oesophageal cancer. This evidence is only based on retrospective case-control studies; cohort studies found no significantly increased risks.

8.7.3 Relationship between nitrate (and nitrite) intake and non-cancer health effects

The JECFA 2003 report

The relationship between nitrate and nitrite intake and non-cancer health effects in humans has been considered by the JECFA at its 59th meeting, which included literature until 2002 (FAO/WHO, 2003a,b). The part of the JECFA report on nitrate intake and non-cancer health effects can be summarized as follows.

A number of studies were performed to determine whether there are associations between nitrate intake in drinking-water and insulin-dependent diabetes mellitus, neural tube defects or sudden infant death syndrome. In none of these studies was a hypothesis proposed for the mechanism of an association. Two studies were conducted on the incidence of insulin-dependent diabetes mellitus and nitrate intake via drinking-water. One study in Yorkshire, United Kingdom, suggested a positive association (McKinney *et al.*, 1999). A study in the Netherlands with a larger number of subjects did not show a positive association. The two studies on nitrate intake and neural tube defects also showed no association (van Maanen *et al.*, 2000). In an ecological study in Sweden, a correlation was reported between the nitrate concentration in drinking-water and the occurrence of sudden infant death syndrome; however, no confounding factors were taken into account (George *et al.*, 2001). The JECFA considered that it would be premature to include these observations in its safety assessment (FAO/WHO, 2003a,b).

New studies since the JECFA 2003 report

In an ecological study in Finland, the association between geographical variation of Type 1 diabetes (IDDM) and its putative environmental risk factors, zinc and nitrate, were investigated. The association was evaluated using Bayesian modelling and the geo-referenced data on diabetes cases and population. Neither zinc, nor nitrate, nor the urban/rural status of the area had a significant effect on the variation in incidence of childhood Type 1 diabetes, although there was a tendency to increasing risk of Type 1 diabetes with the increasing concentration of nitrate in drinking water (Moltchanova *et al.*, 2004).

In a case-control study of Mexican American women, the amine-containing (nitrosatable) drug exposure and neural tube defect (NTD)-affected pregnancies were examined in relation to dietary nitrite and total nitrite intake. A total of 184 women with NTD-affected pregnancies and 225 women with normal live births were interviewed, including questions on periconceptional drug exposures and dietary intake. For 110 study participants, nitrate was also measured in the usual source of drinking water. Women who reported taking drugs classified as nitrosatable were 2.7 times more likely to have an NTD-affected pregnancy than women without this exposure (95% CI = 1.4-5.3). The effect of nitrosatable drugs was observed only in women with higher intakes of dietary nitrite and total nitrite. Women within the highest tertile (greater than 10.5 mg/day) of total nitrite were 7.5 times more likely to have an NTD-affected pregnancy if they took nitrosatable drugs (95% CI = 1.8-45.4). The association between nitrosatable drug exposure and NTDs was also stronger in women whose water nitrate levels were higher. The findings suggested that effects of nitrosatable drug exposure on risk for neural tube defects in offspring could depend on the amounts of dietary nitrite and total nitrite intake (Brender *et al.*, 2004).

Drinking water disinfection by-products have been associated with an increased risk for congenital defects including cardiac defects. Using Swedish health registers linked to information on municipal drinking water composition, individual data on drinking water characteristics were obtained for 58,669 women. Among the infants born, 753 had a cardiac defect. The risk for a cardiac defect was determined for ground water versus surface water, for different chlorination procedures, and for trihalomethane and nitrate concentrations. Ground

water was associated with an increased risk for cardiac defect when crude rates were analyzed but after suitable adjustments this excess rate was found to be associated by chlorination procedures including chlorine dioxide (Cedergren *et al.*, 2002).

In a review of maternal exposure to nitrate in drinking water and adverse reproductive and developmental effects, it was concluded that the current literature does not provide sufficient evidence of a causal relationship (Manassaram *et al.*, 2006).

Conclusions regarding nitrate, nitrite and non-cancer health effects in humans

Taken together with the studies that were reviewed in the JECFA report FAO/WHO, 2003a,b), the CONTAM Panel concluded that there is no clear evidence of an effect of nitrate or nitrite on non-cancer health effects. Most of the evidence is based on methodologically weak ecologic studies, and the lack of individual exposure measurement entails little control for confounding by other causes in the reported studies.

9. Risk characterisation

Vegetables contain higher levels of nitrate than other foods and contribute the most to dietary nitrate exposure. Plants have different storage capacities for nitrate with spinach and lettuce often containing more significant amounts, and rucola having the highest. Some assumptions about vegetable consumption were made in Chapter 6, but detailed information at the individual species level is scarce across Europe. There is anecdotal evidence that rucola consumption is especially popular in Italy and increasing. However, no firm data are available. Results reported to EFSA indicated that the coverage of certain species in relation to production method, season, and region left some gaps. It was possible to generalise overall dietary exposure to arrive at average nitrate intakes, however, some uncertainty remains about regional and individual variations.

The CONTAM Panel estimated dietary exposure to nitrate from vegetables by calculating different exposure scenarios (S1 to S5). Scenario S1 is based on a consumption of 400 g vegetables excluding roots and tubers and herbs. This represents a conservative approach as the international dietary recommendation of 400g/day is for the combined consumption of vegetables and fruit. Scenario S2 is based on the potential contribution of potato consumption at the 97.5th percentile to nitrate exposure. Scenario S3 is based on the highest 97.5 percentile of leafy vegetable consumption at the median level of nitrate recorded with spinach, lettuce and rucola consumption as subscenarios. Scenario S4 explored the impact of splitting overall vegetable consumption at the 400 g/day level into the 97.5th percentile consumption level of leafy vegetables and the remainder as mixed vegetables. Finally, scenario S5 is similar to S4 but takes regionally reported concentrations into account.

The scenario calculations presented in Chapter 7 demonstrate that the critical driver for a high dietary exposure to nitrate is not the absolute amount of vegetables consumed but the type of vegetable and its nitrate concentration. There are a number of factors which can alter the amounts of nitrate consumed. On the one hand preparation such as handling, processing and cooking may

go someway to reducing the concentration of nitrate in vegetables, whereas general dietary recommendations encouraging an increase in the consumption of vegetables and fruit could potentially lead to increased nitrate exposure.

Based on the five scenarios presented in Chapter 7 and also shown in Table 17 the calculated exposure ranged from 82 mg/person/day to 457 mg/person/day when no account was taken of other sources or mitigating factors.

The Panel noted that there were no new hazard data that would alter the JECFA 2002 evaluation and used the ADI for nitrate as derived by the JECFA (FAO/WHO 2003a,b). The ADI of 3.7 mg/kg b.w. is equal to 222 mg of nitrate per person per day at a body weight of 60 kg. Although highly variable, dietary exposure to nitrate from sources other than vegetables is estimated to be on average in the range of 35-44 mg/person/day of which some 20 mg/person/day is contributed by water (see Figure 1). The higher end of this range has been added to the nitrate exposure from vegetables in Table 17 in order to estimate total dietary nitrate exposure for comparison with the ADI in Table 19.

Table 19. Comparison of the ADI for nitrate with different vegetable consumption scenarios including estimates of dietary exposure to nitrate from other sources.

	Vegetable consumption g/person/day	Vegetable	Overall median (S5 - highest regional median) nitrate concentration mg/kg [a]					Calculated total exposure mg/person/day-[h]			% of ADI		
			Potato	Spinach	Lettuce	Rucola	Other	A	B	C	A	B	C
Adults													
S1	400	Most					392	201			91		
S2	771	Potato	106					126			57		
S3	133	Leafy		785	1338	4800		148[c]	222[d]	374[e]	67	100	168
S4	133/267	Leafy/most		785	1338		392	253[f]	327[g]		114	147	
S5	133/267	Leafy/most		1745 [b]	2652 [b]		392	381[f]	501[g]		172	226	

[a] See Table 12
[b] Highest regional median
[c] Spinach at 133 g
[d] Lettuce varieties at 133 g
[e] 1/3 of a leafy vegetable mix as rucola (44 g) and 2/3 as lettuce varieties (89 g)
[f] Spinach at 133 g and other vegetables at 267 g
[g] Lettuce varieties at 133 g and other vegetables at 267 g
[h] Including background exposure from sources other than vegetables (44 mg/person/day).

A = spinach
B = all combined lettuce varieties
C = a mix of rucola (1/3) and lettuce varieties (2/3)

The Panel noted that a high-level consumer of "most vegetables" (S1) or of potato (S2) would not exceed the ADI, neither would a high-level consumer of spinach or lettuce varieties by themselves (S3A or S3B). However, by replacing a third of the leafy vegetables by rucola the ADI would be exceeded (S3C). Indeed, due to its high nitrate content consuming more than 47 g of rucola would result in exceeding the ADI without taking into account any other sources of nitrate exposure. In the event that a high-level consumer of vegetables also consumes lettuce varieties at the 97.5th percentile level (i.e. a third of the vegetables consumed as lettuce, or spinach and lettuce) at the highest regional median levels seen during the winter months, the ADI would also be exceeded. Although the average consumer would not exceed the ADI through vegetable consumption, individuals consuming vegetables produced under unfavourable growing

conditions as in scenario S5 would exceed the ADI by approximately two fold. However, there are a number of mitigation factors (such as fruit consumption and processing) which make this an unlikely regular event. A small part of the population that consume only vegetables, particularly leafy vegetable consumption in high amounts as reported by 2.5% of the population in some Member States (that is the 97.5th percentile consumption level) also can exceed the ADI. Overall, the Panel concluded that there would be no appreciable health risk.

The Panel noted that there can be local situations where drinking water may also significantly contribute to the nitrate exposure, particularly at levels close to the regulatory limit of 50 mg nitrate/L.

Population subgroups

Considering that nutritional recommendations of eating 400 g of fruit and vegetables per day are also valid for children and in the absence of actual data for EU, the estimate was made that children could consume approximately half the amount of adults. Thus 200 g of vegetables was considered to be a reasonable figure for children high consumers and correspond to the 95th percentile of consumption in Germany (Richter *et al.*, 2008). This would result in a nitrate exposure of 78 mg/day. In this case, the ADI of 3.7 mg/kg b.w., corresponding to an acceptable nitrate intake of 74 mg/child/day, based on a bodyweight of 20 kg, would be exceeded by 5%. This does not take into account other sources of nitrate exposure for which good data are not available for children. This could also increase if the vegetable intake consists mainly of leafy vegetables. Nevertheless, the CONTAM Panel recognises that up to one half of the vegetable allocation (Gregory *et al.*, 2000; Richter *et al.*, 2008) is likely to be in the form of fruit, which typically contains low levels of nitrate (normally below 10 mg/kg), and thus for the majority of children the nitrate exposure is likely to be below the ADI.
As outlined in chapter 7 vegetarians and vegans do not significantly differ from the general population in their dietary exposure to nitrate.

10. Benefit identification and characterisation

When benefits are discussed one has to differentiate between the physiological effects, the potential beneficial effects of nitrate and its metabolites and the benefits which can, despite the potential risks of high nitrate levels, be attributed to the consumption of vegetables and fruits because of their composition and nutrients.

Vegetable are considered beneficial in human nutrition as a source of fibre, vitamins and trace elements. In addition, vegetables may contain additional bioactive molecules, such as antioxidants which may serve as chemoprotective agents against chronic diseases and cancer. These positive effects are generally acknowledged by nutritionists and physicians, and the consumption of vegetables is therefore promoted in education programs directed to a balanced nutrition.

10.1 Physiological and pharmacological role of nitrate, nitrite and nitric oxide

Endogenous nitrate synthesis occurs through the L-arginine-NO synthase pathway. Endogenous conversion from nitrate is approximately 1 mg/kg b.w. per day for a 70 kg adult (Archer, 2002). Nitric oxide (NO) is produced from the amino acid L-arginine and this reaction is catalyzed by the NO-synthase (NOS) for which 3 different isoforms have been characterized (Lerzynski *et al.*, 2006). The neuronal NOS1 (nNOS) and endothelial NOS3 (eNOS) forms produce ·NO as a signalling molecule and the inducible form (NOS 2, iNOS) mediates primarily host inflammatory response and its expression is up-regulated by a number of pathological and inflammatory conditions. NO is then oxidized to nitrite, which in turn reacts with oxidized haemoglobin to form nitrate and methaemoglobin. Endogenous NO has essential physiological functions, including the control of blood pressure and regional blood flow, and the limitation of adhesion and aggregation of platelets. In the central nervous system (non-adrenergic, non-cholinergic NANC fibres), NO is involved in neurotransmission, long term potentiation and plasticity (memory, appetite, nociception). In the peripheral nervous system, NO plays a role in neurotransmission, for example, in the regulation of gastric emptying and in blood flow regulation associated with penile erection. The vasodilatory effects have been attributed to the NO-dependent increase of cGMP resulting in a decrease in the intra-cellular Ca++ availability. Major therapeutic indications for the use of nitric oxide donors (i.e. nitroprusside and organic nitrovasodilators such as glyceryl trinitrate) are obstructive coronary heart diseases, pulmonary hypertension, pyloric stenosis in children, and erectile dysfunction).

In inflammatory diseases, upregulation of iNOS results in excessive amounts of NO (and associated radical species), which then contribute to the clinical symptoms of inflammation (vasodilatation and formation of oedemas (Schopfer *et al.,* 2003)). In turn, excessive NO is converted into toxic $ONOO^-$ radicals, which contribute to the non-specific host defence mechanisms against numerous pathogens, including bacteria, fungi, protozoa and parasites, and controversially also to tissue damage due to their cytotoxic effects. The key feature of these mechanisms is that protein tyrosine nitration is part of the inflammation process and moves the physiological role of NO towards an oxidative, nitrative and pathological one, depending on the actual tissue concentration. Nitrogen radicals are also effective against tumour cells (Ying and Hofesth (2007).

There is evidence that enteropathogens can survive for a surprisingly long timein acid alone, but the combination of acid and nitrite results in effective killing. This led to the finding that NO and solutions of acidified nitrite, mimicking gastric conditions, have antimicrobial activity against a wide range of organisms including a variety of gastrointestinal pathogens such as Yersinia and Salmonella (Duncan *et al.*, 1995; Dykhuizen *et al.*, 1996; Vallance, 1997; McKnight *et al.*, 1997; 1999). Thus nitrate, ain the form of nitric oxide may play a role in host defence, (Lundberg *et al.*, 2008).

All nitrogen species, including ·NO, nitrite (NO_2 -), and nitrogen dioxide (·NO_2) may lead to increased concentrations of nitrate in the plasma (Schopfer *et al.*, 2003, Lundberg *et al.*, 2004 and 2008, Cui *et al.*, 2006; Wright *et al.*, 2006).

In humans, a large proportion of exogenous nitrate exposure (60 – 80%) arises from the consumption of vegetables and fruits. Nitrate is converted to nitrite in the human saliva (see chapter 9) and both nitrate and nitrite may be absorbed from the gastrointestinal tract. The contribution of these dietary sources to NO formation (which is regulated in tissues by a negative feed back mechanism involving the control of NOS by intracellular calcium-calmodulin) remains currently unknown.

In this context, a recent study hypothesised that the high nitrate content of beetroot juice represented a source of vasoprotective nitric oxide via bioactivation. In healthy volunteers, approximately 3 hours after ingestion of 500 mL of beetroot juice, a dietary nitrate load of 2.9 g/L, a significant reduction of blood pressure was observed (-10.4/8 mm Hg) and this effect was correlated with peak increases in plasma nitrite concentration. In the human forearm, dietary nitrate load prevented endothelial dysfunction induced by an acute ischemic insult and significantly attenuated ex vivo platelet aggregation in response to collagen and ADP. Interruption of the enterosalivary conversion of nitrate to nitrite prevented the rise in plasma nitrite, blocked the decrease in blood pressure and abolished the platelet aggregation inhibition thus confirming that such vasoprotective effects were mediated via nitrite converted from dietary nitrate (Webb *et al.*, 2008).

10.2 Potential beneficial health effects of nitrate, nitrite and metabolites

Nitrate and nitrite are used as food additives particularly for their anti-bacterial properties against the potentially lethal pathogen *Clostridium botulinum,* and good endogenous efficacy against bacterial gastroenteritis (McKnight *et al.*, 1997; 1999; Duncan *et al.*, 1995; Dykhuizen *et al.*, 1996; Vallance, 1997). This should not be considered a direct health benefit of consuming nitrate. EU food law specifies that food shall not be placed on the market if it is unsafe, and that food is deemed to be unsafe if it is considered to be injurious to health[18]. Hence if, for example, cured ham and bacon products were dependent upon the use of nitrate or nitrite to prevent contamination with *C. botulinum*, they could not be legally marketed if they did not contain nitrate or nitrite.

While the maintenance of human physiological activity is essential for normal health, it is not a health benefit *per se*. In consequence while typical dietary exposure to nitrate should not be considered harmful it cannot be considered to be a health benefit just because it has a range of physiological roles.

It may be, that in certain situations, a diet containing nitrate at levels typically within the ADI may beneficially support the body's endogenous nitrate and nitrite 'pools' (Lundberg, *et al.*, 2008).

[18] Regulation (EC) No 178/2002 OJ L 31, 1.2.2002, p.1-24.

10.3 Potential beneficial health effects of fruits and vegetables

Vegetables provide biologically active substances as well as nutrients like pro-vitamin A, vitamin C, calcium, iron, folate, potassium, magnesium, digestible carbohydrates and non-digestible carbohydrates (fibre), protein. A large range of these is listed in an overview provided in the International Agency for Research on Cancer (IARC) handbooks of cancer prevention (IARC, 2003). In addition, vegetables lack saturated fat and trans fatty acids and are low in sodium which confer them beneficial nutritional properties.

The following is a non-exhaustive list of nutrients and "bioactive substances" found in vegetables and fruit (IARC, 2003).

- **Allyl sulfides**
  - Allicin
- **Carotenoids**
  - Alpha-carotene
  - Beta-carotene
  - Beta-cryptoxanthine
  - Lycopene
  - Lutein
  - Zeaxanthine
- **Citric acid**
- **Flavonoids**
  - Anthocyanins
  - Flavanols
    - Cathechins
    - Proanthocyanidins
  - Flavanones
    - Hesperidin
    - Naringenin
    - Neohesperidin
  - Flavones
    - Apigenin
    - Luteolin
  - Flavonoles
    - Quercetine, Rutin
    - Myricetin
    - Kaempherol
    - Isorhamnetin
  - Isoflavones
    - Genistein, genistin
    - Daidzein, daidzin
    - Glycitein, glycitin
    - Biochanin A
    - Coumestrol
    - Formononetin
- **Fiber**
  - Pectin
  - Inulin
- **Pre-biotics Glucosinolates, and breakdown products**
  - Isothiocyanates
  - Indoles
  - sulphoraphane
- **Lignans**
- **Minerals**
  - Potassium
  - Magnesium
- **Phenolic acids**
  - Cinnamic acids
    - Caffeic acid
    - Chlorogenic acid
    - Ferulic acid
    - Para-coumaric acid
  - Ellagic acid
  - Gallic acid
- **Plant sterols**
  - Beta-sitosterol
  - Campesterol
  - Stigmasterol
- **Resveratrol**
- **Salicylates**
- **Terpenes/terpenoids**
  - limonene
- **Vitamins**
  - folate
  - vitamin C
  - B-vitamins
  - Vitamin K
  - Vitamin E
  - Pro-vitamin A

Relationship between vegetables and health

Fruits and vegetables are frequently considered as one category. The recent reports by the WHO are instrumental in describing the relationship between fruits, vegetables and health. Data are typically derived from observational studies rather than intervention studies. Whereas evidence from observational studies can never provide definitive proof, these data are regarded adequate to support a relationship provided that the data are good and the study designs are appropriate.

Recently, the WHO concluded that "non-communicable diseases" (NCDs), i.e., "chronic diseases" such as cardiovascular diseases (CVDs), cancer, obesity and type 2 diabetes, currently kill more people than any other cause. Four lifestyle factors in the epidemiology of these diseases

(poor diet, physical inactivity, tobacco and alcohol use) are of overwhelming importance to public health (WHO, 2002).

Vegetables and fruit are important components of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as CVDs and certain cancers. According to The World Health Report 2002, low fruit and vegetable intake is estimated to cause about 31% of ischaemic heart disease and 11% of stroke worldwide (WHO, 2002). Overall it is estimated by the WHO that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased (see also Lock *et al.*, 2005).

The 2003 Joint FAO/WHO Expert Consultation on diet, nutrition and the prevention of chronic diseases, recommended the intake of a minimum of 400g of vegetables and fruit per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (WHO, 2003b).

The scientific database describing the evidence for the health benefits of fruit and vegetable consumption is large and growing.

The WHO and the World Cancer Research Fund (WCRF) have reviewed the evidence for the impact of fruits and vegetables on the development of the major chronic diseases (cancer, obesity/management of body weight, cardiovascular disease and diabetes), whereas the International Fruit and Vegetable Alliance (IFAVA) summarised data for other diseases.

An itemised overview of the most relevant diet and health relationships for fruits and vegetables and health is provided below. It should be noted that these chronic diseases are linked: diabetes is associated with cardiovascular disease, overweight and obesity is positively associated with cardiovascular disease, diabetes and cancer.

Impact on cancer

In 1997, the WCRF published their first expert report on "Food, Nutrition and the Prevention of Cancer: a global perspective" (WCFR/AICR, 1997). In its conclusions, the WCRF/AICR stated that "The epidemiological and experimental evidence that the recommended diets decrease the risk of cancer is strong and consistent for many sites. Over time, the implementation of one recommendation – consumption of 400 g/day or more of a variety of vegetables and fruits – could, by itself, decrease overall cancer incidence by at least 20%. The evidence is convincing or probable that diets high in vegetables and/or fruits protect against cancers of the mouth and pharynx, oesophagus, lung, stomach, colon and rectum, larynx, pancreas, breast and bladder." The WCRF/AICR has recently published an updated expert report outlining the extent to which food, nutrition, physical activity, and body composition modify the risk of cancer (WCRF/AICR, 2007).

In the 2003 Handbook on Cancer Prevention the WHO/IARC indicated that approximately one in ten cancers in western populations are due to an insufficient intake of vegetables and fruit. The clearest evidence of a cancer-protective effect of eating more fruits is for stomach and oesophageal cancers. Similarly, a higher intake of vegetables probably reduces the incidence of cancer of oesophagus and colon-rectum (IARC, 2003, WCFR/AICR, 2007).

Impact on overweight and obesity

The WHO has published a review on dietary intake of fruits and vegetables and management of body weight (WHO, 2005a).

Short-term intervention studies, studies with an advice to increase consumption of fruits and vegetables, and studies with a dietary advice only (up to 1 year) showed that in general a diet high in fruits and vegetables and low in fat resulted in significant weight loss in males and females

Impact on cardiovascular disease

The WHO has published a review on dietary intake of fruits and vegetables and risk of cardiovascular diseases (WHO 2005b) Data from both intervention and observational studies indicate that the consumption of fruits and vegetables may play an important role in the prevention of ischaemic heart disease and stroke. An estimate of the potential contribution of the increased intake of fruits and vegetables are 26.000 deaths prevented annually in the EU before the age of 65.

In the recent report "Our Food our Health" (RIVM, 2006) it is estimated that the relative risks (RR[19]) for coronary heart disease is 0.8, when comparing high (> 200 g per day) versus low (< 50 g per day) consumption of fruit and vegetables. When refining the relatively risk for different age groups, it was shown that the relative risk is higher in older age groups (RR = 0.86, 70-79 years; RR = 0.91, > 80 years).

Impact on diabetes

The WHO has published a review on dietary intake of fruits and vegetables and risk of diabetes (WHO, 2005b). A small but growing body of evidence links a diet rich in fruits and vegetables with a lower risk of type 2 diabetes mellitus. The available studies support a role for fruits and vegetables independent of other diet and lifestyle factors in the prevention of type 2 diabetes.

Effects of antioxidants in fruits and vegetables

It has been debated whether the beneficial effects of fruits and vegetables can be broken down into their individual constituents. These compounds have been characterised, tested *in vitro*, *in*

[19] A relative risk smaller than 1 (R<1) means a reduced risk; a RR>1 means an increased risk.

vivo in animals and/or in humans. This reductionist science has made it possible to identify how bioactive substances might affect biological processes using more accurate and sophisticated endpoints. However, the CONTAM Panel concurs with the WHO conclusion (WHO, 2003b) that "The benefit of fruits and vegetables cannot be ascribed to a single or mix of nutrients and bioactive substances. Therefore, the food category was included rather than the nutrients themselves…..".
These conclusions are in accord with other recent reviews (Verhagen *et al.*, 2006; Huang *et al.*, 2006; 2007; Bjelakovic *et al.*, 2007 ; and NIH, 2007).

11. Risk/benefit characterisation

Consumption of various food types varies significantly at different population levels according to age, ethnicity, and dietary habits across different regions within the EU. Nevertheless, there is a growing recognition of the effects of diet as a major lifestyle factor. While vegetables can impact health positively the Panel also noted, that there can be risks associated with the consumption of some vegetables *per se* such as from antinutrients or allergens.

Risk-benefit analysis of foods with regard to human health is a developing area and the EU is now sponsoring a number of EU projects to progress the science, tools, methods and implications, Qalibra[20], Beneris[21] and Brafo[22]. This opinion follows the outline proposed by the EFSA Scientific Colloquium on risk-benefits analysis of foods (EFSA, 2007).

The CONTAM Panel concluded overall, that the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail. The Panel recognised that there are occasional circumstances e.g. unfavourable local/home production conditions for vegetables which constitute a large part of the diet, or individuals with a diet high in vegetables such as rucola which need to be assessed on a case by case basis.

12. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to nitrate has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the draft report on "Characterizing and Communicating Uncertainty in Exposure Assessment", which is in preparation to be published as a World Health Organization/International Programme on Chemical Safety (WHO/IPCS) monograph, has been considered (WHO/IPCS, 2007).

[20] http://www.qalibra.eu/
[21] http://www.beneris.eu/
[22] http://europe.ilsi.org/activities/ecprojects/BRAFO/default.htm

According to the guidance provided by the EFSA (EFSA, 2006) the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model, and model input (parameters).

Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference and the Panel prepared a risk assessment including the consideration of the ADI. The uncertainty in the assessment objectives is considered to be negligible.

Exposure scenario / exposure model

Several exposure scenarios have been considered to estimate the exposure to nitrate. All scenarios are based on the raw products. The possible changes of the nitrate content due to processing of the food commodities, such as washing, peeling and/or cooking could not be considered due to lack of representative data. However, overall, the data indicate that processing is likely to reduce nitrate levels and thus the non-consideration of the quantitative impact of food processing on nitrate levels may lead to an overestimation of the exposure.

Model input (parameters)

A number of uncertainties can be identified regarding the selection of parameters, such as characterisation of levels in food commodities and selection of consumption data.

First of all, the samples reported from the Member States differ greatly regarding the number of vegetables tested as well as concentrations determined in the respective products. Moreover, occurrence data on nitrate in fruits are scarce, although the overall tendency is lower than for vegetables. Thus the conservative base case, that the amount of 400 g of fruits and vegetables recommended by WHO is only allocated to vegetables, results in a high uncertainty and probable overestimate concerning the overall exposure to nitrate.

The estimation of European vegetable consumption from the GEMS Food Consumption Cluster Diet database, which is based on national food balance sheets of annual food production as well as import and export for individual countries aggregated into clusters according to similar consumption behaviour, adds another uncertainty to the exposure assessments

The Panel used the ADI as established by international bodies and recognised the inherent uncertainties in using animal data to derive health based guidance values, but acknowledged the in-built conservatism.

There is uncertainty regarding the optimal amount of vegetable consumption for health benefits. The recommendation of WHO have been used in this assessment.

In Table 20 a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk. The magnitude is related to the source of uncertainty and should not be compared/summed from one source to another.

Table 20. Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to nitrate from vegetables.

Sources of uncertainty	**Direction & Magnitude**
Uncertainty due to type of sampling, as samples are mostly collected in order to check for compliance with legal limits and not for monitoring purposes aimed at estimation of human exposure	++ / -[a)]
Uncertainty about the representativeness of most samples concerning, country of origin, size, regional and seasonal differences, specific type of vegetable	++ / --
Estimation of recommended intake of vegetables and fruits based only on vegetables because of lack of representative nitrate level data for fruits	++
Consumption data from only a number of Member States in combination with data from GEMS Food Consumption Cluster Diet database	++ /-
Uncertainties regarding the influence of food processing and/or cooking on the nitrate levels in the processed food	++
Uncertainties regarding the influence of storage on the nitrite level in food	-
Limitations in certain of the toxicological models e.g. rodents to establish health based guidance values	++

[a)] +, ++, +++= these are used in a semi-quantitative way to indicate the potential to cause small, medium or large over-estimation of exposure/risk.
-, --, --- = uncertainty with potential to cause small, medium or large under-estimation of exposure/risk (EFSA, 2006).

The Panel considered the impact of the uncertainties on the exposure to nitrate due to consumption of leafy vegetables and concluded that the exposure scenarios used tend to overestimate the intake of nitrate and that the risk assessment is likely to be conservative i.e. more likely to over- than to underestimate the risk.

CONCLUSIONS

General

- Nitrate is a naturally occurring compound as well as an approved food additive. Nitrate is also used as a fertiliser and consequently can be an environmental contaminant.

- There are different routes of nitrate exposure for humans: endogenous formation, and exogenous exposure from dietary and non-dietary sources.

- The main dietary sources of nitrate are vegetables, preserved meat and drinking water.

Exposure assessment

- In total nearly 42,000 analytical results originating from 21 European countries concerning 92 vegetable varieties were considered in this assessment.

- While there is a large variation in the median concentration of nitrate in different vegetables from 1 mg/kg (peas and Brussels sprouts) to 4,800 mg/kg (rucola), green leafy vegetables have been shown consistently to have the highest levels.

- Several factors such as light intensity, storage, processing and/or cooking of vegetables influence nitrate concentrations.

- Different exposure scenarios calculated on the basis of the recommended daily intake of 400 g vegetables and fruit for adults (including vegetarians), but all consumed in the form of vegetables, showed that it is not the amount of vegetable eaten but the type of vegetable and its nitrate content that is the critical driver for consumer exposure.

- Exposure to nitrate from eating 400 g of mixed vegetables per day at typical median nitrate concentrations was estimated to be 157 mg/day.

- Nitrite is also found in vegetables but generally at much lower concentrations than nitrate. These levels are not a major direct contributor to human exposure compared with endogenous formation from nitrate.

Hazard characterisation

- A toxicological endpoint of concern for nitrate is nitrosamine formation and the potential for tumour formation. However, when nitrate is consumed in a normal diet containing vegetables, other bioactive substances concomitantly consumed, such as the antioxidant vitamin C, may inhibit the endogenous formation of nitrosamines.

- Epidemiological studies relating to nitrate and human cancer risk do not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk.

- Evidence that high intake of nitrite might be associated with increased cancer risk is equivocal.

- No new data were identified that would require a revision of the ADI values of 0-3.7 mg/kg body weight for nitrate and 0-0.07 mg/kg b.w. for nitrite as reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives in 2002.

Risk characterisation

- Dietary exposure estimates showed that the ADI for nitrate would not be exceeded by an adult eating 400 g of mixed vegetables. However, high level consumers, of vegetables grown under unfavourable local production conditions may exceed the ADI approximately two fold. In these calculations the nitrate concentrations were not corrected for mitigation factors e.g. fruit consumption and processing and may overestimate exposure. Overall, the Panel concluded that there would be no appreciable health risk.

- Consumption of more than 47 g of rucola at the median nitrate concentration would lead to an excursion above the ADI without taking into account any other sources of nitrate exposure.

Benefit Characterisation

- A range of physiological roles of nitrate and its metabolites are increasingly appreciated as a result of recent research. However, the extent to which exogenous nitrate contributes to human physiology in healthy individuals remains to be established.

- There is a general consensus that a balanced diet high in vegetables and fruit confers significant health benefits in terms of a reduction of the risk for a range of diseases.

Risk/benefit characterisation

- Overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail.

- The Panel recognised that there are occasional circumstances e.g. unfavourable local/home production conditions for vegetables which constitute a large part of the diet, or individuals with a diet high in vegetables such as rucola which need to be assessed on a case by case basis.

Recommendations

- There is a need for research into the factors that influence nitrate and nitrite concentrations and alterations during productions, storage and processing.

- Member States should submit individual analytical data on those crops regularly found to contain high levels of nitrate.

- Some vegetables such as rucola can make a disproportionate contribution to overall nitrate exposure, and hence changing dietary habits need to be closely monitored.

- Continued efforts to progress methodology for the risk-benefit analysis of foods remain a high priority.

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DOCUMENTATION PROVIDED TO EFSA

Committee of Professional Agricultural Organisations in the EU (COGECA), Belgium. Letter including a paper from the UK lettuce growers.

Food Standards Agency (FSA), United Kingdom. Letter.

National Farmers Union (NFU), United Kingdom. Letter including a paper from the National Farmers Union on behalf of the UK lettuce growers.

Mechelse Veilingen, Belgium. Letter including scientific articles.

Section Nationale Salades. Email including scientific articles.

LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
CBT	Childhood brain tumours
CEN	European Committee for Standardization
CONTAM	Panel on Contaminants in the Food chain
CVDs	Cardiovascular diseases
EFSA	European Food Safety Authority
GAP	Good agricultural practices
GC	Gastric cancer
GI	Gastrointestinal
IARC	International Agency for Research on Cancer
IFAVA	International Fruit and Vegetable Alliance
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	Limit of detection
ML	Maximum level
NDMA	N-nitrosodimethylamine
NHL	Non-Hodgkin lymphoma
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect-level
NOS	Nitric oxide synthetase
NTD	Neural tube defects
OC	Ooesophageal cancer
OR	Odds ratio
RRs	Relative risks
SCF	Scientific Committee for Food
SIRs	Standardized incidence ratios
TOR	Terms of reference
WCRF	World Cancer Research Fund
WHO	World Health Organization
WHO/IPCS	World Health Organization/ International Programme on Chemical Safety

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Dietary Nitrates, Nitrites, and N-Nitroso Compounds and Cancer Risk: A Review of the Epidemiologic Evidence

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Experimental animal studies have shown N-nitroso compounds (NOC) to be potent carcinogens. Epidemiologic evidence of the carcinogenic potential of dietary NOC and precursor nitrates and nitrites in humans remains inconclusive with regard to the risk of stomach, brain, esophageal, and nasopharyngeal cancers. Inadequate available data could obscure a small to moderate effect of NOC.

Introduction

Various N-nitroso compounds (NOC) have been found to be carcinogenic to multiple organs in at least 40 animal species including higher primates.[1] The cellular and molecular changes induced by some NOC in animals have been shown to be very similar to those in human tissues.[2] In addition to exposure to preformed NOC (e.g., tobacco use, certain occupational environments, diet),[2,3] humans are exposed to nitrogen-containing compounds and nitrosating agents, which can react in vivo to form NOC. Nitrate, nitrite, and nitrosating agents can also be synthesized endogenously in reactions mediated by bacteria and macrophages.[2] The efficacy of certain vitamins as nitrosation inhibitors provides a plausible explanation of epidemiologic findings that have shown a protective effect of fruit and vegetable consumption against various malignancies.[4,5] Despite extensive information regarding carcinogenicity of NOC in animals, there have been few analytic studies investigating the risk in humans, and what is available is limited to case-control studies. This paper reviews the epidemiologic evidence relating estimated dietary intake of NOC, nitrates, and nitrites (and some examples of individual foods rich in these substances) with the risk of stomach, brain, esophageal, and nasopharyngeal cancers.

Vegetables usually contribute 75–80% of the total daily intake of nitrate, with high levels in lettuce, spinach, celery, beetroot, turnip greens, etc. The nitrate concentration of drinking water varies widely depending on the source (high concentrations in private water supplies), season, and proximity to arable land. Nitrate and nitrite are often added as preservatives to processed (cured) meat, meat products, and fish. Nitrites are also found naturally in some grains and vegetables. Nitrosodimethylamine has been found in various processed meats (salted, cured, or smoked) and fish and in beer.[3,6,7]

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Stomach Cancer

Large differences in the incidence of stomach cancer exist worldwide. The highest incidence rates are found in Japan and China; Switzerland and France have intermediate rates; and North America and Greece have the lowest rates. The continuous decline of stomach cancer rates over the past several decades and the results of migrant studies suggest a predominant etiologic role for external environmental factors generally believed to be dietary. A recent review by the American Institute for Cancer Research[8] considers consumption of diets high in vegetables and fruits and low in salt and the use of refrigeration for perishable foods as the most effective means of preventing stomach cancer. An important established nondietary cause of stomach cancer is infection with the *Helicobacter pylori* bacterium. Various other potential risk factors such as high consumption of grilled and barbecued meat and fish and cured meats are discussed. The stomach is an established site for NOC carcinogenesis in animals.[8,9]

Of the six case-control studies that estimated dietary intake of nitrate and its association with stomach cancer risk, three revealed non–statistically significant results[10–13] whereas the other three studies[14–16] found a significant inverse association with stomach cancer. The methods and results of these studies are described in Table 1.[10–17] In the Canadian study of Risch and coworkers,[15] 246 cases of stomach cancer were compared with 246 population-based controls matched by age, sex, and area of residence. The apparent protective effect (odds ratio [OR]=0.66, 95% confidence interval [CI]=0.54–0.81) of dietary intake of

Table 1. Case-Control Studies on Dietary Nitrate, Nitrite, and N-Nitroso Compounds and Risk of Stomach Cancer

Reference and Number of Cases	Nitrate	Odds Ratio (95% CI)[a]	Nitrite	Odds Ratio (95% CI)[a]	N-Nitrosodi-methylamine	Odds Ratio (95% CI)[a]
Canada						
Risch et al.[15] (*n*=246)	↓	0.66 (0.54–0.81)[b]	↑	2.61 (1.61–4.22)[c]	NS	0.94 (0.14–6.13)[b]
Four areas in Italy						
Buiatti et al.[10] (*n*=1016)	NS	0.9 (0.7–1.2)[d]	NS	1.2 (0.8–1.8)[e]		
Palli et al.[11] (*n*=923)						
Cardia	NS	1.1 (0.6–2.3)[f]	NS	0.9 (0.3–2.7)[g]		
Other gastric cancer	NS	0.7 (0.6–1.0)[f]	NS	1.2 (0.8–1.9)[g]		
Sweden						
Hansson et al.[12] (*n*=338)	NS	0.97 (0.60–1.59)[h]	NS	1.22 (0.82–1.81)		
Spain						
González et al.[16] (*n*=354)	↓	0.45 (*p* trend = 0.007)[i]	NS	1.28 (*p* trend = 0.377)[i]	Nitrosa-mine ↑	2.09 (*p* trend = 0.007)[i]
Greater Milan area						
La Vecchia et al.[14] (*n*=723)	↓	0.64 (0.43–0.97)[j]	NS	1.12 (0.78–1.59)[j]		
La Vecchia et al.[17] (*n*=746)					↑	1.37 (1.1–1.7)[k]
France						
Pobel et al.[13] (*n*=92)	NS	0.76 (0.38–1.50)[l]	NS	0.88 (0.44–1.79)[l]	↑	7.00 (1.85–26.46)[l]

Note: CI=confidence interval. NS=statistically not significant. ↑=statistically significant direct association. ↓=statistically significant inverse association.

[a] Highest intake level vs. lowest.

[b] Adjusted for food consumption and ethnicity.

[c] Model simultaneously includes dietary fiber, nitrite, chocolate, carbohydrates, no refrigeration, total food consumption, ethnicity.

[d] Adjusted for nondietary variables and kilocalories.

[e] Adjusted for kilocalories, nondietary variables, protein, ascorbic acid, ß-carotene, α-tocopherol.

[f] Adjusted for caloric intake, age, sex, area, place of residence, migration from the South, socioeconomic status, familial history, Quetelet index.

[g] Adjusted for [f] plus protein, ascorbic acid, β-carotene, α-tocopherol.

[h] Multivariate analysis including age, gender, ascorbic acid, β-carotene, α-tocopherol, nitrates in the same model.

[i] Adjusted for total calories.

[j] Estimates for multiple logistic regression equations including terms for age, sex, education, family history of gastric cancer, body mass index, total energy intake, β-carotene, ascorbic acid, folate, methionine, nitrate, and nitrite, respectively.

[k] Adjusted for age, sex, education, family history of gastric cancer, combined food score index, intake of β-carotene, vitamin C, and total calories, nitrate, and nitrite.

[l] Adjusted for age, sex, occupation, total calorie intake.

nitrate was reversed to a nonsignificant positive association (OR=1.63, 95% CI = 0.904–3.04) when vitamin C intake was taken into account (both calculated from the consumption of 21 vegetables).

In a study carried out in selected areas of four regions in Spain, including 354 cases of gastric adenocarcinoma and 354 hospital controls matched by age, sex, and area of residence,[16] González et al. concluded that the observed reduced risk for nitrates (adjusted for total calories) might just be an indicator of vegetable consumption, known to be associated with a reduced risk of stomach cancer.[3]

Of the six case-control studies that estimated nitrite intake, five[10–14,16] showed no significant association with stomach cancer risk. In the aforementioned study of Risch et al.,[15] a direct association was observed (OR=2.61, 95% CI=1.61–4.22, adjusted for dietary fiber, chocolate, carbohydrates, no refrigeration, total food consumption, and ethnicity). The same held true for the data of La Vecchia et al.[17,18] in which the interaction between methionine and nitrites was considered. Compared with subjects with low methionine (< 1.5 mg/day) and low nitrite intake (<2.7 mg/day), the OR was 2.45 (95% CI=1.9–3.2) in the high methionine (>1.9 mg/day) and high nitrite (≥2.7 mg/day) stratum. Measures of methionine and nitrite intake were derived from a selected number of foods only. These data were derived from an ongoing case-control study conducted in the Greater Milan area between 1985 and 1993.[17,18]

Of the four studies that estimated NOC intake,[13,15–17] three showed a statistically increased risk with high intake of N-nitrosodimethylamine (NDMA).[13,16,17] In the case-control study conducted by Pobel et al. in Marseilles, France[13], the OR for the third versus the first tertile of intake was 7.00 (95% CI=1.85–26.46, adjusted for age, sex, occupation, and total calorie intake). The wide confidence intervals probably reflect the small number of cases (*n*=92) and give an imprecise estimation of the OR. Only dietary exposure to NDMA was assessed, although it may not be

representative of the whole group of preformed nitrosamines in food. In the study by González et al.,[16] it was suggested that high consumption of a protective factor, such as vitamin C, neutralizes the increased risk observed with consumption of preformed nitrosamines (OR=2.09 in the highest quartile, adjusted for total calories). In the study by La Vecchia et al.,[17] the multivariate OR for the highest NDMA intake tertile was 1.37 (95% CI=1.1–1.7) including age, sex, education, family history of gastric cancer, combined food score index, intake of β-carotene, vitamin C, total calories, nitrite, and nitrate. No information on *H. pylori* in cases and controls was available, although *H. pylori* antibody prevalence has not been shown to correspond to high-risk areas of gastric cancer in Italy.

Table 2[19–22] shows the results of four case-control studies of foods rich in nitrate, nitrite, and N-nitroso compounds and risk of stomach cancer. In 1985, Correa et al.[19] presented results of 391 stomach cancer cases and an equal number of hospital controls with a wide variety of clinical conditions matched by race, sex, and age, with both groups being inhabitants of southern Louisiana. Smoked foods (OR 1.70, 95% CI=1.01–2.87) and homemade sausages or home-cured meats (OR 2.32, 95% CI=1.10–4.87) were associated with a statistically significant increased risk for stomach cancer in blacks but not whites after adjustment for sex, respondent status, income, and duration of smoking. Multiple comparisons made while examining factors related to stomach cancer increased the probability of a statistically significant result owing to chance alone.

A case-control study of 564 stomach cancer patients and 1131 population-based controls was conducted to evaluate reasons for the high rates of stomach cancer in Linqu, in northeast China.[20] Risk of stomach cancer was increased by 50% among families with "moldy grain" supplies (several species of fungus can reduce nitrate to nitrite[23]). These results, however, were adjusted only for sex, age, and income.

Boeing et al.[21] investigated 143 cases of stomach cancer in a high-risk area and a low-risk area for stomach cancer in Germany and compared them with 579 controls who were patients or visitors from the same hospitals matched for age and sex. They reported a nonsignificant negative association with nitrate from food items in a univariate analysis, but this association changed to a nonsignificant positive association in multivariate analysis. Furthermore, the authors reported a significantly elevated risk for users of well water compared with those who used central water supplies at some time during the lifecycle (OR=2.26, 95% CI=1.19–4.28). These results were adjusted only for home meat smoking, years of refrigerator use, age, sex, and hospital. No data were available on water constituents, but analyses from other countries have shown that private water sources can contain considerable amounts of nitrate.

Conversely, Rademacher et al.[22] found no association with nitrate levels in water (central or private water sources) and cancer risk. This large study compared 1268 stomach cancer deaths in Wisconsin residents with an equal number of deaths from other causes matched by sex, year of birth, year of death, Wisconsin birth, and Wisconsin residency at the time of death. There were some weaknesses inherent in the study. The results were not adjusted for other potential confounders, such as ethnicity and dietary habits. Moreover, the place of residence listed on the death certificate (hospitals or nursing homes excluded) was assumed to be the source of the subjects' nitrate exposure

Table 2. Case-Control Studies on Foods Rich in Nitrates, Nitrites, or N-Nitroso Compounds and Risk of Stomach Cancer

Reference and Number of Cases	Dietary Variable	Comparison	Association	Odds Ratio (95% CI)	Population
Correa et al.[19] (*n*=391)	Smoked foods	Above median intake	↑	1.70 (1.01–2.87)[a]	Louisiana
	Homemade sausage or cured meats	Vs. lower intake	↑	2.32 (1.10–4.87)[a]	
You et al.[20] (*n*=564)	"Sour pancakes"	Daily vs. <daily	NS	1.3 (1.0–1.6)[b]	Shandong, China
	Moldy grain	Yes vs. never	↑	1.5 (1.2–2.0)[b]	
Boeing et al.[21] (*n*=143)	Well and central water	Vs. central water only	↑	2.17 (1.38–3.39)[c]	Germany
	Well water		↑	2.26 (1.19–4.28)[c]	
	Nitrate from food items	Quintile 5 vs. quintile 1	NS	1.26 (0.59–2.70)[d]	
Rademacher et al.[22] (*n*=1268)	Private well	Vs. public water	NS	1.09 (0.82–1.47)[e]	Wisconsin
	>10.0mg/L NO3-N in public sources	Vs. less, or private	NS	1.50 (0.12–18.25)[e]	

Note: CI=confidence intervals. NS=statistically not significant. ↑=Statistically significant direct association.
[a] For blacks after adjustment for sex, respondent status, income, duration of smoking.
[b] Adjusted for sex, age, family income.
[c] Adjusted for smoking of meat at home, years of refrigerator, age, sex, hospital.
[d] Adjusted for vitamin C, carotene, calcium, age, sex, hospital.
[e] Crude odds ratio.

via drinking water for at least 20 years before death (the latent period of most carcinogenic exposures). It was concluded that random misclassification error, a major problem in retrospective studies in which past exposure must be estimated, could have existed. Exposure misclassification of this type would tend to bias the OR toward unity.

In general, N-nitroso compounds, found in cured meats, salted foods, etc., may be related to the risk of stomach cancer, but the available epidemiologic evidence is insufficient[8] to confirm this hypothesis.

Brain Tumors

The most common types of brain tumors are astrocytoma, medulloblastoma, ependymoma, glioblastoma, and meningioma. The age curve of these tumors shows a peak during the first decade of life followed by peaks in adults, except for medulloblastoma, which is rarely observed in adults, and meningioma, which is less prevalent in children than in adults. Brain tumors account for about one in five childhood cancers. Increased incidence has been noted in many countries, mainly in adults, and this may reflect diagnostic improvement. Very little is known about the etiology of brain tumors. One postulated risk factor that has been the subject of investigation is exposure to NOC and precursor nitrates and nitrites, some of which are nervous system carcinogens in animals, especially when exposure occurs transplacentally.[9,24,25]

Table 3[24–30] shows the results of studies of NOC and brain tumors. Five case-control studies[24–28] investigated maternal dietary exposure to nitrosamines during pregnancy. One study considered dietary intakes by children.[27] Two studies investigated all childhood brain tumors combined,[26,27] despite the fact that different brain tumors may have different etiologies.

In Los Angeles County, Preston-Martin et al.[26] questioned mothers of 209 young brain tumor patients and mothers of 209 population-based controls (matched by sex, race, and birth year) about experiences of possible etiologic relevance that they had during pregnancy, including frequency of consumption of cured meats. Results suggested an etiologic role for cured meats (ORs=1.2 for moderate, 2.3 for high versus low, intake; p trend=0.008) and other NOC-containing substances in childhood brain tumors.

In a small Canadian case-control study[27] that compared children's consumption of cured meats before diagnosis (>1 serving/week versus ≤1) in 74 cases and 138 age- and sex-matched population controls, no association was observed.

Newer studies have concentrated on a single type of brain tumor in children. Gestational and familial risk factors were investigated for their association with astrocytoma in a case-control study of 163 pairs (matched by age, race, and telephone exchange) that was performed in Pennsylvania, New Jersey, and Delaware.[25] The researchers observed a significant trend showing more frequent consumption of cured meats in mothers of astrocytoma patients compared with control mothers. However, the association was present only among more highly educated mothers (OR=6.8, 95% CI=1.8–26.3).

Conversely, a study by Bunin et al.[24] showed no elevated risk with frequent maternal consumption of cured meats (quartile 4 versus quartile 1) and primitive neuroectodermal tumor in children. The 166 case patients had a primitive neuroectodermal tumor in the brain diagnosed before the age of 6 years between 1986 and 1989 and were registered with the Children's Cancer Group in North America. The 166 controls (matched by age and race) were selected by random-digit telephone dialing. A parallel study of astrocytic glioma in children (155 case-control pairs) was conducted by the same investigators and interviewers using the identical questionnaire.[28] No significant association between cured meat consumption during pregnancy and risk of astrocytic glioma (adjusted for income level) was shown. Misclassification owing to difficulty in reporting diet during a pregnancy up to 6 years in the past might have contributed to the negative results.

Two investigations concentrated on brain tumors in adults (Table 3). Burch et al.[29] studied 215 adult males (25–80 years of age) diagnosed in southern Ontario between 1979 and 1982 and an equal number of hospital-based controls matched by sex, area of residence, marital status, year of birth, date of diagnosis, and date of death. The study included many dead cases. Thus, the quality of dietary data was poor because of the large number of proxy respondents. The investigators observed elevated risks for reported consumption of spring water (OR=4.33, 95% CI=1.24–15.2) and wine (OR=2.14, 95% CI=1.28–3.60) (ever versus never) for brain tumors in general. Although spring water and wine consumption are consistent with a role for NOC in the etiology of brain tumors, for several other factors related to this hypothesis (e.g., consumption of various processed meat and fish products), no association was observed.

Preston-Martin et al.[30] investigated employment histories and other suspected risk factors of 272 men ages 25–69 with a primary brain tumor first diagnosed during 1980–1984 in Los Angeles County and of 272 age- and race-matched neighbor controls. Separate analyses were conducted for 202 glioma pairs and 70 meningioma pairs. No significant direct association between NOC-rich beer, wine, and hard liquor consumption (ever consumed at least once a month versus less) and risk of gliomas or meningiomas in males was observed.

In summary, although some studies point to weak associations, the available data provide little support for the hypothesis that N-nitroso compounds are involved in the etiology of brain tumors.

Case-Control Studies on Dietary Intake of Nitrates, Nitrites, and N-Nitroso Compounds (or the Corresponding Foods) and the Risk o[f]

:e and [illegible] Cases	Brain Tumor	Dietary Variable	Comparison	Association	Odds Ratio (95%CI)	Population
[illegible] et al.[26]	"Brain tumors"	Intake during pregnancy				Los Angeles Coun[ty]
)		Cured meats	High vs. lower	↑	2.3[a]; p trend=0.008	children < 25 yea[rs]
al.[27]	"Brain tumors"	Cured meats (child)	>1×/week vs.≤1	NS	1.13 (0.551–2.31)[b]	Southern Ontario
		Beer (pregnancy)	Ever vs. never	↑	3.53 (1.16–10.8)[b]	cases ≤ 19 years
t al.[25]	Astrocytoma	Intake during pregnancy	Yes vs. no	NS	1.9 (0.9–4.2)[a]	Cases < 15 years,
)		Cured meat	Frequency	↑	p trend=0.04	New Jersey, Dela[ware]
		Highly educated mothers	High	↑	6.8 (1.8–26.3)[a]	Pennsylvania
		Less educated mothers	High	NS	1.2 (0.4–3.8)[a]	
al.[24]	Primitive neuro-ectodermal tumor	Intake during pregnancy				U.S., Canadian
)		Nitrate	Quartile 4 vs. quartile 1	NS	0.54 (-)[c]	children < 6 year[s]
		Nitrite		NS	1.06 (-)[c]	
		Nitrosamines		NS	1.55 (-)[c]	
		Cured meat		NS	1.10 (0.60–2.03)[a]	
al.[28]	Astrocytic glioma	Intake during pregnancy				U.S., Canadian
)		Cured meats	Quartile 4 vs. quartile 1	NS	1.7 (0.8–3.4)[d]	children < 6 year[s]
		Nitrite		NS	1.3 (0.7–2.6)[d]	
		Nitrate		NS	0.7 (0.3–1.4)[d]	
		Dimethylnitrosamine		NS	0.8 (0.4–1.6)[d]	
al.[29]	"Brain tumors"	Spring water	Ever vs. never	↑	4.33 (1.24–15.2)[a]	Southern Ontario
)		Wine	Ever vs. never	↑	2.14 (1.28–3.60)[a]	adults (25–80 ye[ars])
Martin et al.[30]	Gliomas (G)	Beer	>1×/month vs. less	NS	G: 0.7 (0.5–1.2)[a]	Los Angeles Coun[ty]
)	Meningiomas (M)			↓	M: 0.4 (0.1–0.9)[a]	men (25–69 year[s])
		Wine		NS	G: 0.7 (0.5–1.1)[a]	
				NS	M: 0.7 (0.3–1.4)[a]	
		Hard liquor		NS	G: 1.3 (0.8–1.9)[a]	
				NS	M: 0.7 (0.3–1.4)[a]	

confidence intervals. NS=not statistically significant. ↑=statistically significant direct association. ↓=statistically significant inverse association.
lds ratio.
l for age at diagnosis.
l [illegible]ood components and supplements.
l for income level.

Esophageal Cancer

The highest incidence rates of esophageal cancer are found in the so-called Asian esophageal cancer belt, which stretches from Russia and Turkey to eastern China. In Europe, the highest rates are found in France. It is more common in males in America and Europe, whereas in the high-risk Asian belt, as the incidence rises the proportional male predominance declines. Correlation studies suggest that the causes might not be the same in all countries. Although alcohol and tobacco may account for as much as 90% of esophageal cancer in some Western populations, these factors appear to play a minor role in areas with the highest incidences, such as inland Asia.[23] No clear explanation is available regarding the etiology of Asian belt esophageal cancer. Various hypotheses have been proposed, including diets low in fruits and vegetables, diets inadequate in numerous vitamins and minerals, physical trauma to the esophagus (e.g., by the high temperature of ingested food and beverages), consumption of foods rich in N-nitroso compounds, nitrates, or nitrites (e.g., salted and pickled vegetables), and consumption of moldy foods.[8,9,23,31,32]

Correspondingly, several ecologic studies in China have shown an association between indices of exposure to N-nitroso compounds or precursors (e.g., consumption of pickled vegetables) and esophageal cancer mortality.[32] However, a large-scale case-control study in the high-risk area of Linxian of 1244 patients with cancer of the esophagus or gastric cardia and 1314 population-based age- and sex-matched controls did not detect any increase in risk associated with the use of pickled vegetables.[33] The authors of the study suggested that the control group might have included a large proportion of subjects with chronic esophagitis and dysplasia. These may be premalignant lesions and may therefore share risk factors with esophageal cancer.

In a cohort study[34] conducted in the same area, a total of 1162 subjects from the analytic group of 12,693 developed esophageal cancer over the 15-year follow-up period. Results indicated that traditional or suspected risk factors for esophageal cancer, such as smoking and alcohol use, and consumption of pickled vegetables and moldy food were not risk factors for esophageal (including gastric cardia) cancer, but the findings were adjusted only for age and sex.

These results were reiterated in a hospital-based case-control study of Hu et al.[35] that included 196 cases and 392 controls with other (nonneoplastic, nonesophageal) diseases (matched by sex, age, and area of residence) that was carried out in a low-risk area of northeast China. Salt, salt-preserved foods, and pickled vegetables were not associated with an increased risk of esophageal cancer (Table 4[23,31,33–36]). The results were adjusted for alcohol intake, smoking, income, and occupation. Imperfect recollection of diet in the past might have led to random misclassification, and a limited range of exposure to the investigated foods might have decreased the chances to ascertain significant associations.

Conversely, a case-control study conducted by Cheng et al.[36] of 400 Hong Kong Chinese cases and 1598 age- and sex-matched controls (800 hospital and 798 general practice) showed a direct association between pickled vegetable consumption and esophageal cancer risk. In the analysis, consumption of pickled vegetables was divided into six categories: < once/year, < once/month, 1–3 times/month, 1–3 times/week, 4–6 times/week, and daily and more, with the consumption of < once/year being the reference group. The corresponding ORs adjusted for age, level of education, and birthplace were 2.07 (95% CI=0.93–4.60), 1.64 (95% CI=0.84–3.17), 2.35 (95% CI=1.20–4.61), 5.96 (95% CI=2.4–14.77), and 18.10 (95% CI=4.84–67.71). The test for trend was statistically significant. It should be noted, however, that the last two categories of pickled vegetable consumption consisted of only 20 cases and 23 controls and 14 cases and 5 controls, respectively, thus yielding imprecise estimations of the ORs. In a multivariate model including age, consumption of alcohol, smoking, consumption of green leafy vegetables and citrus fruits, preference for hot drinks or soups, place of birth, education, and domestic dining versus eating out during early adult life, the OR of consumption of pickled food daily or more versus < once/year was 13.12 (95% CI=2.57–66.93), i.e., remained statistically significant.

Similar results were reported in the case-control study by Wang et al.[23] that was conducted in two areas of Shanxi (Yangcheng and Linfen), in north central China. The study included 326 cases and 396 population-based controls matched by sex, age, and residence. Esophageal cancer risk tended to increase with increased intake ("sometimes, often" versus "never, rarely") of moldy foods and pickled vegetable juice (high nitrite concentrations[37]). The results (Table 4) were adjusted only for age, gender, and farming/nonfarming occupation, and in Yangcheng the category "sometimes, often" of the consumption of pickled vegetable juice consisted of only seven cases and two controls.

A population-based case-control study (902 cases, 1552 age- and sex-matched controls) by Gao et al.[31] of esophageal cancer in Shanghai found, after adjusting for smoking, alcohol consumption, and other potential confounders (see Table 4), that consumption of preserved vegetables, fermented bean curd (which may be contaminated with mycotoxins), and salty and deep-fried foods was linked to increased risk, but these results were not consistently statistically significant in subgroups.

With regard to drinking water, Yu et al.[34] showed in their retrospective cohort study in Linxian a significant reduction in risk associated with drinking well instead of surface water. The relative risk of 0.83 (95% CI=0.69–0.99)

Table 4. Case-Control Studies on Dietary Intake of Foods Rich in Nitrates, Nitrites, or N-Nitroso Compounds and Risk of Esophageal Cancer in the "Asian Belt"

Reference and Number of Cases	Dietary Variable	Comparison	Association	Odds Ratio (95% CI)	Population
Li et al.[33] (n=1244)	Pickled vegetables	More than 1×/day vs. never in 1970, high risk communes	NS (M) NS (F)	0.9 (0.6–1.3)[a] 1.1 (0.7–1.7)[a]	Linxian, China
Wang et al.[23] (n=326)	Pickled vegetable juice	Sometimes/often vs. never/rarely	Yangcheng ↑ Linfen ↑	3.6 (1.1–18.4)[b] 11.6 (6.3–21.6)[b]	Shanxi, China Yangcheng: high risk Linfen: moderate risk
	Moldy foods	Sometimes/often vs. never/rarely	Yangcheng ↑ Linfen ↑	5.0 (2.6–9.9)[b] 6.5 (3.7–11.2)[b]	
Cheng et al.[36] (n=400)	Pickled vegetables	Daily or more vs. <1×/year	↑	13.12 (2.57–66.93)[c]	Hong Kong Chinese
Yu et al.[34] (retrospective cohort study) (n=1162)	Pickled vegetables Regular moldy food use Water use	Regular vs. occasional/never Yes vs. no Well vs. pond or river	NS NS ↓	RR: 1.03 (0.92–1.15)[d] RR: 1.09 (0.95–1.24)[d] RR: 0.83 (0.69–0.99)[d]	Linxian, China
Hu et al.[35] (n=196)	Pickled cabbage Fermented soy paste	Quartile 4 vs. quartile 1 Quartile 4 vs. quartile 1	NS NS	0.7 (0.4–1.2)[e] 0.7 (0.4–1.3)[e]	Northeast China: low-risk area
Gao et al.[31] (n=902)	Salty foods	Very salty vs. not salty	NS (M) ↑(F)	2.27 (0.89–5.77)[f] 3.81 (1.27–11.50)[f]	Shanghai, China
	Cured foods	Frequently vs. never/seldom	NS (M) NS (F)	1.25 (0.86–1.84)[f] 1.33 (0.84–2.13)[f]	
	Preserved				
	Salty vegetables	Quartile 4 vs. quartile 1	NS (M) ↑(F)	1.2 p trend=0.23[g] 1.7 p trend<0.05[g]	
	Fermented bean curd	Quartile 3 vs. quartile 1	↑(M) ↑(F)	1.4 p trend<0.05[g] 1.8 p trend<0.01[g]	
	Vegetable moldy, dried	Quartile 3 vs. quartile 1	NS (M) ↑(F)	1.2 p trend=0.26[g] 1.9 p trend<0.01[g]	

Note: CI=confidence interval. M=male. F=female. NS=statistically not significant. ↑=statistically significant direct association. ↓=statistically significant inverse association. RR=relative risk.

[a] Adjusted for age and (for males) smoking.

[b] Adjusted for age, gender, farm/nonfarm occupation.

[c] Adjusted for age, consumption of alcohol, smoking, consumption of green leafy vegetables, consumption of citrus fruits, preference for hot drinks or soups, whether had meals at home or eating out during early adult life, place of birth, education.

[d] Adjusted for age and sex.

[e] Adjusted for alcohol intake, smoking, income, and occupation.

[f] Adjusted for age, education, birthplace, tea drinking, cigarette smoking, alcohol drinking (men), consumption of preserved foods, vegetables, and fruit.

[g] Adjusted for age, education, birthplace, tea drinking, cigarette smoking, alcohol drinking (men).

was adjusted only for sex and age. The elevated risk observed for surface water might have been related to a higher nitrate content in that water, but no data on the nitrate concentrations of the two water sources were given. No association between water source and esophageal cancer risk was observed in the previously mentioned case-control study of Li et al.[33] that was carried out in the same area (results not shown).

The recent review by the American Institute for Cancer Research[8] concluded that ecologic evidence supported by experimental data suggests that exogenous dietary N-nitrosamine exposure and endogenous N-nitrosamine formation possibly increase the risk of esophageal cancer.

Nasopharyngeal Cancer

Although tumors of the nasopharynx are rare in most countries, they are prevalent in Chinese residents of Southeast Asia, Arabs in North Africa, and Inuit populations of Mongoloid origin in Canada, Greenland, and Alaska. Known and suspected causes are genetic factors, Epstein-Barr virus (EBV), inhaled substances, smoking, and diet, especially Cantonese salted fish.[8,9]

Case-control studies in southern China, Malaysia, and Hong Kong demonstrated an association between the consumption of salted fish, especially during weaning, and the risk of nasopharyngeal cancer.[38–40] The methods and the results of these and other studies are described in Table 5.[38–45] Ning et al.[41] reported on a case-control study performed in a low-risk region of China (Tianjin) with data from 100 cases of nasopharyngeal cancer and 300 neighborhood controls (matched by age, sex, and race). Exposure to salted fish (ever versus never) was significantly associated with an increased risk of nasopharygeal cancer (OR=2.2, 95% CI=1.3–3.7). The following characteristics of exposure to salted fish independently contributed to the increased risk: earlier age at first exposure, increasing duration and frequency of consumption, and steaming of fish rather than frying, grilling, or boiling it. Results were not adjusted for other risk factors. In a separate analysis, a significant increased risk was observed for the consumption of salted shrimp paste and salted fish when adjusted for each other and for carrot consumption, but not for infection with Epstein-Barr virus and other factors.[41]

A more recent case-control study of Zheng et al.[42] (88 nasopharyngeal cancer cases, 176 age-, sex-, and neighborhood-matched controls) was conducted in Znagwu County, Guangxi, China, and was part of the study of Hubert et al.[46] This multivariate analysis (including the use of wood fuel, consumption of herbal tea, and a sociodemographic score) found a significantly increased risk (OR=3.8, 95% CI=1.5–9.8) for the consumption of salted fish in rice porridge before the age of 2 years. Because subjects provided data on their diet from almost 30 years previously these results may be affected by recall bias.

Additionally, Sriamporn et al.[43] conducted a case-control study with data from 120 nasopharyngeal cancer cases and the same number of hospital-, age-, and sex-matched controls in northeast Thailand, a region that shows an intermediate risk for this neoplasm. The consumption of sea-salted fish at least once a week versus never in adult life was a significant risk factor for nasopharyngeal cancer (OR=2.5, 95% CI=1.2–5.2, adjusted for alcohol, cigarette consumption, occupation, education, and area of residence). Again, EBV infection as a potential confounder was not assessed.

In the recent review by the American Institute for Cancer Research,[8] the overall evidence that diets high in Cantonese-style salted fish increase the risk of nasopharyngeal cancer is considered convincing. Salted fish has a high level of secondary amines. These amines are believed to interact with nitrite salts used as preservatives and lead to the formation of N-nitroso compounds, which are possibly organotrophic for the nasopharynx.[9] This has been demonstrated in vivo by Yu et al.,[47] who induced malignant nasal cavity tumors in rats fed salted fish.

Rates of nasopharyngeal cancer comparable to those in Southeast Asia have been reported in Inuit populations in Canada, Alaska, and Greenland and in Arabs of North Africa. Cantonese Chinese, Maghrebian Arabs, and Eskimos were compared in anthropologic studies by Hubert et al.[46] It should be noted that the diet of Maghrebian Arabs, for example, is very different from that of Chinese and does not include salted fish. The conclusion of Hubert's study was that traditional preserved food preparations could be the common factors linking these groups. Laboratory analyses of food samples from south China, Macao, Tunisia, and Greenland revealed the presence of volatile nitrosamines.[48] In a third step of the study by Hubert et al.,[46] case-control studies in Tunisia and in China tested the hypotheses based on these data. The results suggested that consumption in early youth of salted and preserved foods other than salted fish, such as fermented fish sauce, salted shrimp paste, moldy bean curd, and two kinds of preserved plums, was also associated with an increased risk of nasopharyngeal cancer.[40,44,45]

Conclusions

N-Nitroso compounds (NOC) are potent carcinogens in animal studies.[1] Epidemiologic evidence of dietary NOC and precursor nitrates and nitrites as human oncogenic agents remains inconclusive. In assessments of the human health risks of dietary exposure to nitrate, nitrite, and NOC, it is important to recognize that the analysis of the exposure is particularly complex.[13] Many NOC have been detected in foods, but only N-nitrosodimethylamine is well studied. Nitrate, nitrite, and NOC concentrations in food products, in addition, can vary widely for the same food or for drinking water from different sites. The accurate

Table 5. Case-Control Studies of Foods Rich in Nitrates, Nitrites, or N-Nitroso Compounds and Risk of Nasopharyngeal Cancer

Reference and Number of Cases	Dietary Variable	Comparison	Association	Odds Ratio (95% CI)	Population
Armstrong et al.[38] (n=100)	Consumption during childhood				
	Salted fish	Daily vs. never	↑	17.4 (2.7–111.1)[a]	Malaysian Chinese
Yu et al.[39] (n=250)	During weaning				
	Salted fish	Ever vs. never	↑	7.5 (3.9–14.8)[a]	Hong Kong
	At age 10 years				
	Salted fish	≥ 1×/week vs. rarely	↑	37.7 (14.1–100.4)[a]	
Yu et al.[44] (n=128)	During pregnancy				
	Salted fish	Weekly vs. rarely	↑	3.1 (1.1–8.8)[b]	Yulin Prefecture, China
	During weaning				
	Salted fish	Yes vs. no	↑	2.6 (1.2–5.6)[b]	
	Salted duck eggs		↑	5.0 (1.2–21.0)[b]	
	Salted mustard green		↑	5.4 (1.2–23.8)[b]	
	Chung choi		↑	2.0 (1.3–3.2)[b]	
	Age 1–2 years				
	Fermented black bean paste	Weekly vs. rarely	↑	4.6 (1.8–11.4)[b]	
	Fermented soy bean paste		↑	3.6 (1.6–8.1)[b]	
	Salted fish, dried fish, salted mustard green		NS		
	At age 10 years				
	Dried fish		↑	6.4 (1.6–26.8)[b]	
	Salted fish, fermented black or soy bean paste, chung choi, salted mustard green		NS		
Yu et al.[40] (n=306)	During pregnancy				
	Salted fish	Daily vs. rarely	↑	2.2 (1.1–4.6)[a]	Guangzhou, China
	During weaning				
	Salted fish	Yes/no	↑	2.1 (1.2–3.6)[a]	
	Exposure during ages 1–2				
	Salted fish	Weekly vs. rarely	↑	2.0 (1.1–3.6)[a]	
	Fermented fish sauce		NS	p trend=0.07[a]	
	Salted shrimp paste		NS	p trend=0.06[a]	
	Moldy bean curd		↑	p trend=0.02[a]	
	Around age 10 years				
	Salted fish	Daily vs. rarely	↑	2.1 (1.2–3.6)[a]	
	Fermented fish sauce		NS	p trend=0.86[a]	
	Salted shrimp paste		↑	p trend=0.02[a]	
	Moldy bean curd		NS	p trend=0.85[a]	
	Kind of preserved plum		↑	p trend=0.01[a]	
	3 years ago				
	Salted fish	Daily vs. rarely	NS	1.8 (0.9–3.6)[a]	
Jeannell et al.[45] (n=80)	Preserves/condiments (child)				
	Stewing mixture	Yes/no	↑	8.6 (1.7–43.5)[c]	Tunisia
	Snack of harissa	≥Once/month vs. <once/month	↑	4.2 (1.1–16.7)[c]	
Ning et al.[41] (n=100)	Salted fish	Ever vs. never	↑	2.2 (1.3–3.7)[a]	Tianjin, China
	Age at first exposure (yr)				
	≥21		NS	1.5 (0.7–3.3)[a]	
	11–20		NS	1.9 (0.9–4.0)[a]	
	1–10		↑	2.6 (1.5–4.6)[a]	
	Duration of consumption (yr)				
	1–10		NS	1.6 (0.9–3.1)[a]	
	11–20		↑	2.8 (1.4–5.4)[a]	
	≥21		↑	2.8 (1.4–5.6)[a]	
	Frequency of consumption (at age 10 years)				
	Yearly		NS	1.6 (0.8–3.2)[a]	
	Monthly		↑	3.5 (1.6–7.4)[a]	
	Weekly/daily		↑	6.7 (2.2–20.7)[a]	
	Cooking method (at age 10 years)				
	Steamed		↑	4.2 (2.2–8.3)[a]	
	Other (frying, grilling, boiling)		NS	1.6 (0.8–3.2)[a]	
	Salted shrimp paste	Weekly/daily vs. none	↑	3.2 (p=0.007)[d]	
Sriamporn et al.[43] (n=120)	Adult consumption				
	Salted fish	≥1×/week vs. none	↑	2.5 (1.2–5.2)[e]	Northeast Thailand
Zheng et al.[42] (n=88)	Before age 2 years				
	Salted fish in rice porridge	Monthly/weekly vs. rarely	↑	3.8 (1.5–9.8)[f]	Guangxi, China

Note: CI=confidence interval. NS=statistically not significant. ↑=statistically significant direct association.
[a] Crude values.
[b] Adjusted for subject's sex and age.
[c] Matched logistic analysis adjusted for the living conditions score.
[d] Adjusted for consumption of salted fish and carrot consumption.
[e] Adjusted for alcohol, cigarettes, occupation, education, area of residence.
[f] Adjusted for use of wood fuel, consumption of herbal tea, sociodemographic score.

recall of food is another problem in case-control studies.[3] Cohort studies are less prone to this bias, but no prospective study has been reported. Moreover, endogenous production of NOC and its precursors may be a more important source of exposure than exogenous intake. In addition, where exposure appears to have a small effect, the amount of uncontrollable confounding inherent in analytic epidemiologic studies is about as large as the most plausible effect.[49] Finally, many of the studies discussed in this review did not estimate dietary intake of NOC, nitrates, and nitrites, but used dietary intake of individual foods rich in these substances as a proxy measure. Exposure misclassification may explain some of the negative study results.

In summary, although no firm epidemiologic evidence had been found linking stomach, brain, esophageal, and nasopharyngeal cancers to dietary intake of nitrate, nitrite, and NOC, an association cannot be ruled out. The strongest evidence points to an increased risk of nasopharyngeal and esophageal cancer in subjects exposed to high dietary NOC levels.

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United States
Environmental Protection
Agency

Consideration of Other Regulatory Revisions in Support of the Second Six-Year Review of the National Primary Drinking Water Regulations

Office of Water (4607M)
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Table of Contents

List of Acronyms and Abbreviations

AMG	Alternative Monitoring Guidelines
ASDWA	Association of State Drinking Water Administrators
BAT	Best Available Technology
CCR	Consumer Confidence Report
CFR	Code of Federal Regulations
CWS	Community Water System
EPA	United States Environmental Protection Agency
EPTDS	Entry Point to the Distribution System
FR	*Federal Register*
GWUDI	Ground Water under the Direct Influence of Surface Water
HAA5	Haloacetic Acids
LCR	Lead and Copper Rule
LT2ESWTR	Long-Term 2 Enhanced Surface Water Treatment Rule
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg/L	Milligrams per Liter
MRL	Method Reporting Limit
NAS	National Academy of Sciences
NPDWR	National Primary Drinking Water Regulation
NTNCWS	Non-transient, Non-community Water System
OCCT	Optimal Corrosion Control Treatment
OECA	Office of Enforcement and Compliance Assurance
OGC	Office of General Counsel
OGWDW	Office of Ground Water and Drinking Water
OPEI	Office of Policy, Economics, and Innovation
PN	Public Notification
POE	Point-of-Entry
POU	Point-of-Use
PQL	Practical Quantitation Limit
PWS	Public Water System
SDWA	Safe Drinking Water Act
SMCL	Secondary Maximum Contaminant
SNC	Significant Non-Complier
TCR	Total Coliform Rule
Stage 2 DBPR	Stage 2 Disinfectant and Disinfection Byproducts Rule
TNCWS	Transient Non-Community Water System
TSC	Technical Support Center
TT	Treatment Technique

UCMR	Unregulated Contaminant Monitoring Regulation
UIC	Underground Injection Control
V&E	Variance and Exemption
VOC	Volatile Organic Compound

1.0 Introduction and Background

1.1 Purpose of the Six-Year Review

Under the Safe Drinking Water Act (SDWA), as amended in 1996, the U.S. Environmental Protection Agency (EPA) must periodically review existing National Primary Drinking Water Regulations (NPDWRs) and, if appropriate, revise them. Section 1412(b)(9) of SDWA states:

> *The Administrator shall, not less often than every 6 years, review and revise, as appropriate, each national primary drinking water regulation promulgated under this title. Any revision of a national primary drinking water regulation shall be promulgated in accordance with this section, except that each revision shall maintain, or provide for greater, protection of the health of persons.*

EPA completed and published the results of its first Six-Year Review (Six-Year Review 1) on July 18, 2003 (68 *Federal Register* [FR] 42908, USEPA, 2003a) after developing a systematic approach, or protocol, for the review of NPDWRs. EPA has applied the same protocol with some refinements to the second Six-Year Review of NPDWRs (Six-Year Review 2) (USEPA, 2009).

To facilitate the regulatory review of a large number of NPDWRs, EPA performs a series of analyses at the beginning of each review cycle, intended to target those NPDWRs that are the most appropriate candidates for revision. During each review cycle, EPA reviews the following key information and/or factors to determine whether regulatory revisions are possible and appropriate: health risk assessments; analytical methods and treatment technology assessments; occurrence and exposure analyses; and other regulatory revisions (such as implementation-related issues).

1.2 Purpose of the Review of "Other Regulatory Revisions"

In addition to the review of the maximum contaminant level goals (MCLGs), maximum contaminant levels (MCLs), and treatment techniques (TTs) components of the NPDWRs, EPA considers whether other regulatory revisions might be needed, such as system monitoring and reporting requirements, as part of Six-Year Review process. For the Six-Year Review 2, EPA utilized the protocol established during Six-Year Review 1 for evaluating which implementation issues to consider (USEPA, 2003b). EPA's protocol focused on items that were not already being addressed, or had not been addressed, through alternative mechanisms (e.g., as a part of a recent or ongoing rulemaking). In addition to this limitation, EPA considered potential implementation-related revisions if they:

1) Represented a potential change to an NPDWR, as defined under section 1401 of SDWA[1];
2) Were "ready" for rulemaking – that is, the problem to be resolved had been clearly defined and specific option(s) had been formulated to address the problem under the current regulatory framework; and
3) Would clearly improve the level of public health protection; and/or provide a meaningful opportunity for cost savings (either monetary or burden reduction) while not lessening public health protection.

[1] The subject of the Six-Year-Review, as specified in section 1412(b)(9) of the SDWA, is "each national primary drinking water regulation," as defined under section 1401 of SDWA.

2.0 Issues Identified by the EPA/State Workgroup

To gather input regarding implementation-related concerns and help the Agency identify the top one or two issues for Six-Year Review 2, EPA requested that the Association of State Drinking Water Administrators (ASDWA) form a workgroup of member States and primacy agencies. In the fall of 2007, ten member States agreed to participate and confer with EPA on a joint EPA/State workgroup (see Appendix A for a list of States and EPA offices that participated in the workgroup). In the initial meeting, EPA asked participating States to work towards identifying their top one or two implementation-related issues and formulate potential solutions that States would be willing to implement and EPA could feasibly address under existing regulatory frameworks. EPA also provided participating States with an overview of the guidelines used for Six-Year Review 1, to help States better understand the scope of the review process.

To compile an initial list of possible issues, the workgroup requested feedback from all States. The feedback from States resulted in a list of 22 possible issues. ASDWA then asked States from the workgroup to rank each of the issues as high, medium, or low priority. Eight of the ten workgroup members responded. Total scores used for ranking the issues were calculated by assigning the following values: high priority - 3 points; medium - 2 points; and, low - 1 point, and then tallying the scores for each issue. The list of all 22 issues identified during the workgroup process is presented in Appendix B; issues are listed in order of highest to lowest priority score, and their actual score totals are provided in the "State/Workgroup Priority Score" column of the table. Concurrent with the ranking process, EPA used the factors listed on page 1 (Section 1.2) to evaluate whether the issues were: (1) best addressed through technical assistance, guidance, or other mechanisms[2], (2) outside the scope of this Six-Year Review[3], or (3) within the scope of the this Six- Year Review and could possibly be addressed by regulatory action. These groupings are reflected in the "Findings" column of Appendix B. Although the primary purpose of the workgroup was to identify the top issues that were within the scope of this NPDWR review, EPA attempted to provide assistance during the workgroup meetings by having Agency experts discuss some of the items that fit within the technical assistance/guidance categories.

Based on issue rankings and determinations of how issues were best addressed, the workgroup narrowed the list of 22 down to 4 issues. Of these four items, three appeared to be within the scope of the Six-Year Review, and EPA agreed that an information or fact sheet might be appropriate for the fourth item, which pertains to the need for clarification of public notification (PN) requirements for fluoride (see Section 3.1 for summary of this "non-Six-Year Review" issue). The EPA/State workgroup agreed that public comment via the FR would provide additional insight on the national scope of these issues (i.e., Are the issues isolated to a few States/systems or more widespread?); the importance of these issues to other States, as well as the public water systems (PWSs); and ideas for potential resolutions. This additional input could further assist in identifying the top one or two issues that should be considered for regulatory revision.

[2] An example of an item identified by the States that was better addressed through technical assistance is the issue of false positive analytical test results (e.g., for phthalates). EPA addressed this concern with States during the course of the workgroup meetings, offering direct technical assistance from laboratory experts at EPA's Technical Support Center (TSC) in Cincinnati, Ohio, and noting that TSC is a resource for any laboratory with questions regarding methods issues, with contact information available at: http://www.epa.gov/esd/tsc/tsc.htm. EPA provided additional information and technical experts to suggest possible solutions for each of the issues raised by the States (see Appendix B).

[3] An example of an issue that was "outside of the scope of the Six Year Review" was a concern raised related to the Unregulated Contaminant Monitoring Regulation (UCMR). Because the UCMR is not an NPDWR, as defined under section 1401 of SDWA, it was therefore not within the scope of the Six-Year Review.

The following sections of this document provide background and summary information regarding the three issues that were within the scope of an NPDWR review, as well as the fourth item (PN requirements for fluoride) for which EPA is considering some form of information or fact sheet. Potential resolutions discussed by State workgroup members are also summarized. EPA recognizes that some of the potential resolutions suggested by the State workgroup members may need to be better defined prior to any potential revision that the Agency might consider. Issues that fall within the scope of an NPDWR revision for the current review effort include:

- Section 2.1 – Change the location of monitoring for nitrate/nitrite.
- Section 2.2 – Reduce the monitoring frequency for ground water systems with historically low levels of nitrate/nitrite.
- Section 2.3 – Revise the monitoring requirements for non-community water systems in light of the potential health risks associated with chronic contaminants.

2.1 Change the Monitoring Location for Nitrate/Nitrite

Issue Description

States in the workgroup expressed concern that nitrification within the distribution system may be a growing issue[4]. And while the extent or cause has yet to be fully examined, there is some concern that nitrification is occurring in water systems that have adopted chloramines as a disinfection treatment option and potential exceedances above the MCL for nitrate/nitrite may go undetected at the current sampling location[5].

See Appendix B for the original tracking notes on this issue.

Potential Resolution(s) Suggested by State Workgroup Members

To address this concern, the State workgroup members suggested moving the location of the nitrate/nitrite sampling point. This would either be somewhere other than the entry point to the distribution system (EPTDS), or the system could maintain the existing EPTDS sample location and add additional sampling points in the distribution system[6]. The State workgroup members also posed several potential options for the frequency of sampling. First, sampling for nitrate/nitrite could be done on the same schedule for bacteria under the Total Coliform Rule (TCR). Second, the samples for nitrate/nitrite could be taken together with samples for disinfection byproducts under the Stage 2 Disinfectant and Disinfection Byproducts Rule (Stage 2 DBPR). Lastly, the sampling

[4] Nitrification is a microbial process by which reduced nitrogen compounds (primarily ammonia) are sequentially oxidized to nitrite and nitrate. See www.epa.gov/safewater/disinfection/tcr/pdfs/whitepaper_tcr_nitrification.pdf for additional information on nitrification.

[5] The health effects technical review identified new information on developmental effects of nitrate and nitrite, as well as data regarding its carcinogenicity, which may indicate the need to update the Agency's risk assessment. In light of this information, EPA is considering nitrate and nitrite as potential candidates for new health effects assessments. If new assessments are initiated, EPA does not expect that they will be completed in the time frame of the current Six-Year Review cycle. When the new assessments are completed EPA will be able to determine the potential impacts on the MCLG, MCL, and/or monitoring requirements, and the most appropriate timing for any potential revisions.

[6] The monitoring framework suggested by the workgroup is consistent with the monitoring requirements for six nitrosamine compounds in the second Unregulated Contaminant Monitoring Regulation for Public Water Systems (UCMR 2) (72 FR 367 (USEPA, 2007)). Under that rule, some PWSs are required to sample both at the entry point to the distribution system and within the distribution system at the point of maximum residence time.

could occur at particular points in the distribution at a frequency to be determined. The Agency indicated that additional data on nitrate/nitrite occurrence within the distribution system would be needed to determine if the issue is State-specific or national in scope. Although Texas provided some monitoring data[7], workgroup members agreed that taking public input on this topic might generate the data needed to better define the scope of the issue.

Although this flexibility was not addressed during workgroup deliberations, EPA notes that 40 CFR 141.23(a)(2) allows surface water systems discretion to locate the sampling point in the distribution system if that is more representative of the source after treatment.[8]

2.2 Reduce the Monitoring Frequency for Ground Water Systems with Historically Low Levels of Nitrate/Nitrite

Issue Description

The workgroup discussed the possibility of monitoring relief for ground water systems with many years of nitrate/nitrite results that were well below the existing MCL. States in the workgroup asserted that because nitrate/nitrite levels do not fluctuate significantly over time in stable ground water sources, reduced monitoring would not decrease public health protection, and would lower monitoring costs for these systems and reduce the State tracking burden. Under the current rule, States cannot issue waivers for nitrate monitoring, and no water system can conduct nitrate monitoring less frequently than annually[9].

EPA published the current NPDWR for nitrate on January 30, 1991 (56 FR 3526 (USEPA, 1991)) (40 CFR 141.62), establishing an MCL of 10.0 milligrams per liter (mg/L), and the requirement that all PWSs must monitor for nitrate at each EPTDS. The federal regulations required nitrate monitoring to begin in 1993 at a quarterly frequency for community water systems (CWSs) and non-transient, non-community water systems (NTNCWSs) with surface water or ground water under the direct influence of surface water (GWUDI) sources, and annually for all other systems including transient non-community waters systems (TNCWSs). If monitoring results identified nitrate occurrence at less than one-half the MCL, CWSs and NTNCWSs with surface water or GWUDI sources could reduce quarterly monitoring to annual monitoring (to occur in the quarter that previously yielded the highest nitrate monitoring result). All other systems were required to remain on annual monitoring.

See Appendix B for the original tracking notes on this issue.

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[7] Subsequent to these initial discussions, the Minnesota Department of Health has also performed studies and gathered some data on nitrification in the distribution system.

[8] 40 CFR 141.23(a)(2) states: Surface water systems shall take a minimum of one sample at every entry point to the distribution system after any application of treatment or in the distribution system at a point which is representative of each source after treatment (hereafter called a sampling point) beginning in the initial compliance period. The system shall take each sample at the same sampling point unless conditions make another sampling point more representative of each source or treatment plant.

[9] Note that the Federal regulation at 40 CFR 141.23(a)(1) and (2), and 141.23(e) provide for more flexibility for reduced nitrite monitoring. Systems were only required to monitor for nitrite once during the initial compliance period, or between 1993 and 1995. Systems with analytical results that are less than one-half the MCL of 1.0 mg/L conduct continued monitoring at a frequency specified by the State. The federal regulations do not require these systems to monitor for nitrite again.

Potential Resolution(s) Suggested by the State Workgroup Members

The workgroup members discussed several regulatory revision options to address these ground water systems that have historically low levels of nitrate/nitrite. The potential options included:

- Revisions to Monitoring Frequency -- The State workgroup members suggested a reduced monitoring frequency of 3, 6, or 9 years. These monitoring schedules are consistent with the reduced monitoring provisions of the existing standard monitoring framework. The States suggested that some other frequency could be established as well, and noted that even a two-year monitoring frequency would help lessen the monitoring burden.
- Monitoring or Trigger Level to Qualify for Reduced Monitoring -- The State workgroup members also discussed options for a new trigger (nitrate concentration) level that would qualify systems to begin this new reduced monitoring schedule. The new trigger level would be some fraction of the MCL (e.g., one-half the MCL), the practical quantitative limit/method detection limit (or some other descriptor of detection), or some other appropriate trigger level.
- Duration of Meeting Trigger Level to Qualify for Reduced Monitoring -- States discussed how long a system would need to meet this trigger level to be allowed to begin reduced monitoring. One proposal was to use a 3-, 6-, or 9-year period consistent with the standard monitoring framework. Another proposal was to use a 5-, 10-, or 15-year option.

State workgroup members also discussed the need for a waiver option that would give States the discretion to allow systems to monitor less. EPA recommended that States consider a non-regulatory option for monitoring relief. States that adopt EPA's Alternative Monitoring Guidelines (AMG) (established in 1997 under section 1418(b) of SDWA) would have the flexibility to reduce nitrate sampling for ground water systems from an annual to a biennial (every other year) requirement. However, to adopt the AMG, States would need to undergo a full rule adoption process, and many States felt that this process was too cumbersome. States expressed that there is some hesitation to adopt AMG because in many cases, adoption of the AMG could also place system sampling out of sync with their standard chemical monitoring schedule. States indicated that they would prefer some type of regulatory revision for these ground water systems instead of using the AMG.

2.3 Revise the Monitoring Requirements for Non-Community Water Systems in Light of the Potential Health Risks Associated With Chronic Contaminants

Issue Description

The workgroup raised two concerns about balancing public health protection, and use of limited financial resources associated with non-community water system monitoring. In the case of NTNCWSs, EPA requires monitoring for contaminants that pose a health risk from chronic exposure (other than radionuclides). The workgroup suggested that some of this monitoring may not reflect the best use of limited resources. In light of the probability and magnitude of health threats, some monitoring requirements for these systems may be insufficient, and others may be excessive. However, the workgroup was also concerned that EPA does not require monitoring for these contaminants at TNCWSs, and that this may pose a potential public health risk. Though some States would have the flexibility to require additional monitoring for TNCWSs, this is not an option

in States with statutes that prohibit them from applying regulations more stringent than those specified by EPA.

See Appendix B for the original tracking notes on this issue.

Potential Resolution(s) Suggested by the State Workgroup Members

The State workgroup members posed three potential options for regulatory revision related to monitoring by non-community water systems. The first option was to revise all contaminant rules to include additional monitoring requirements for TNCWSs, as well as radionuclide monitoring for NTNCWSs. The second option was to review existing regulated contaminants and include TNCWS monitoring requirements based on the relative health risk from chronic exposure. The third option was to develop general language that would apply to all contaminant rules, giving States the discretion to require additional monitoring for contaminants that pose chronic exposure risks and can have acute effects at elevated levels potentially found at TNCWSs. Most States in the workgroup tended to prefer the third option since it offered the most flexibility for States. For some of these options EPA would need to evaluate whether sufficient occurrence and exposure data are available for TNCWSs and NTNCWSs to assess the need for revised monitoring strategies.

3.0 Other Issues

3.1 Public Notification Requirements for Fluoride

The fourth item that was identified by the State workgroup members pertains to the need for clarification of PN requirements for fluoride. Although PN is not within the scope of the Six-Year Review because it is not an NPDWR as defined by SDWA section 1401, EPA agreed this item could be addressed outside the review process, possibly through some form of information or fact sheet to clarify the PN requirements for fluoride.

Currently, PWSs that exceed the fluoride MCL of 4.0 mg/L are required to notify their customers within 30 days of the exceedance. If a PWS exceeds the fluoride Secondary MCL (SMCL) of 2.0 mg/L, they are required to notify their customers within 12 months of the exceedance. The States voiced concerns about (1) the confusion that occurs between the different PN requirements for the MCL and the SMCL, and (2) the timeliness of the PN requirement for the SMCL.

The workgroup indicated that waiting 12 months to notify customers of an exceedance of the SMCL does not adequately protect young children from dental fluorosis during a critical stage of tooth enamel development. The participating States requested that EPA consider regulatory revisions to clarify the PN requirements and better reflect the health and aesthetic implications of each. EPA noted that PN requirements are not within the scope of an NPDWR review though agreed that a fact or information sheet may be useful to clarify any confusion.

The Agency is updating its evaluation of the relative contribution of drinking water to total fluoride exposure considering the contributions from dental products, foods, pesticide residues, and other sources such as ambient air and medications. After the Agency completes and publishes peer reviewed versions of these assessments, it will be able to determine the potential impacts on the MCLG, MCL, and/or the SMCL, and associated PN requirements.

See Appendix B for the original tracking notes on this issue.

References

USEPA. 1991. National Primary Drinking Water Regulations; Synthetic Organic Chemicals and Inorganic Chemicals; Monitoring for Unregulated Contaminants; National Primary Drinking Water Regulation Implementation; National Secondary Drinking Water Regulations; Final Rule. *Federal Register.* Vol. 56, No. 20, p. 3526. January 30, 1991.

USEPA. 2003a. National Primary Drinking Water Regulations; Announcement of Completion of EPA's Review of Existing Drinking Water Standards. *Federal Register.* Vol. 68, No. 138, p. 42908. July 18, 2003.

USEPA. 2003b. *EPA Protocol for Review of Existing National Primary Drinking Water Regulations.* EPA Report 815-R-03-002. Final. June 2003.

USEPA. 2007. Unregulated Contaminant Monitoring Regulation (UCMR) for Public Water Systems Revisions; Final Rule. *Federal Register.* Vol. 72, No. 2, p. 367. January 4, 2007.

USEPA. 2009. *EPA Protocol for the Second Review of Existing National Primary Drinking Water Regulations (Updated).* EPA Report 815-B-09-002. October 2009.

Appendices

Appendix A: Review of Implementation Issues – States and EPA Offices Participating in the Workgroup

Appendix B: Second Six-Year Review of National Primary Drinking Water Regulations - Summary of Initial List of 22 Issues Identified by Working Group

Appendix C: Summary of the January 18, 2008 Discussion on Issues Use of POU/POE Devices

Appendix A: Review of Implementation Issues – State and EPA Offices Participating in the Workgroup

ASDWA/States	
ASDWA (Liaison to EPA)	Nebraska
Delaware	New Jersey
Idaho	New York
Minnesota	Oregon
Missouri	Texas
North Carolina	
EPA Offices	
Office of Enforcement and Compliance Assurance (OECA)	Office of Ground Water and Drinking Water (OGWDW)
Office of General Counsel (OGC)	Office of Policy, Economics, and Innovation (OPEI)

Appendix B: Second Six-Year Review of National Primary Drinking Water Regulations - Summary of Initial List of 22 Issues Identified by Working Group

Issue[1]	Description	State Workgroup Priority Score	Findings *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
Monitoring and Reporting Violations	**More clearly distinguish between monitoring and reporting violations so that the actual significance of each type of violation is known:** Under the current violation tracking process, a water system that samples and reports the results a day late looks as bad as a system that does not collect any samples at all. This may have ramifications on the status of systems as Significant Non-Compliers (SNCs). There may be a solution to this issue that does not require a regulatory change.	21	Outside the scope of this review effort; best handled through technical assistance.	

Issue[1]	Description	State Workgroup Priority Score	**Findings** *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
Chem/Rad Rules (*Arsenic and Uranium MCLs)*	**States would like assistance in determining cost effective methods for dealing with chem/rad wastes:** The new MCLs for arsenic and uranium are causing problems for some very small systems (e.g., < 50 connections). The problem is not in the treatment, but in the disposal of the treatment waste. This is a significant implementation issue that has never been adequately addressed by EPA.	21	Outside the scope of this review effort; best handled through guidance.	EPA noted during workgroup discussions that many guidance documents/training materials related to treatment/disposal costs have been developed for the arsenic and radionuclides regulations. The following websites link to the various webcasts and/or other sources of information for these rules: www.epa.gov/safewater/dwa/rules.html www.epa.gov//safewater/radionuclides/compliancehelp.html www.epa.gov/safewater/arsenic/compliance.html In a follow up email, EPA provided a weblink (www.npdespermits.com/sparrc/) to a simulation tool entitled "Software Program to Ascertain Radionuclide Residual Concentrations (SPARRC)." This tool can be used to estimate quantities and concentrations of radium and uranium in water treatment plant residuals (for selected treatment technologies using a mass balance approach). In this version, EPA also incorporated a disposal cost estimating tool for a few technologies in SPARRC, and included default unit costs based on national average cost information. EPA has used available case study data to validate the mass balance calculations and compared outputs with another radionuclide mass balance model. The users can estimate quantities and costs for radionuclide-contaminated residuals given user-defined inputs for influent water quality and treatment operation parameters.

Issue[1]	Description	State Workgroup Priority Score	**Findings** *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
LT2ESWTR	**Provide regulatory relief for small system cryptosporidium requirements:** Early implementation of 40 CFR 141.701(a)(4)(i) has shown that one sample after months and months of low readings can trigger cryptosporidium monitoring by small systems. Some relief seems appropriate.	20	Outside the scope of this review effort since the NPDWR was just revised and published in 2006; best handled through other mechanisms.	Current Long-Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) offers flexibility. EPA has been collecting data on turbidity, *E. coli*, and cryptosporidium for large water systems; this information may help to evaluate monitoring requirements for small systems.
Lead and Copper Rule *(extend OCCT plan timeframe)*	**Modify the Lead and Copper Rule to extend the optimal corrosion control treatment (OCCT) plan submission time frame to 12 months and then allow 18 months after that to install treatment:** Very few systems have been able to complete the study and submit plans under the existing timeframes.	18	Outside the scope of this review effort since the NPDWR was recently revised and published in 2007.	EPA is currently evaluating issues for the long-term revision effort and this item is best handled by referring it to this effort.
Nitrate-Nitrite monitoring	**Revise current nitrate/nitrite monitoring requirements to more appropriately reflect chloramination considerations:** Many water systems are adopting disinfection with chloramines as a treatment option to reduce levels of disinfection byproducts. Nitrification may be an issue but currently source water monitoring is the only requirement for nitrate and nitrite. A potential health impact could go undetected. One State (Texas) noted that they were developing data on occurrence in distribution systems but does not have these data yet.	18	Within the scope of considering for this review effort; best handled through regulatory revision or clarification.	Regulatory revision may be needed to change the location of the sampling point for monitoring. Guidance could also be considered as part of the solution; would need information/data to better understand the conditions that lead to nitrification. See section 2.1 of this document for further discussion.

Issue[1]	Description	State Workgroup Priority Score	**Findings** *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
Non-Community System Monitoring	**Review and revise, as appropriate, monitoring requirements applicable to non-community water systems in light of the potential health risks associated with each:** NTNCWSs monitor for chronic contaminants but TNCWSs do not. There may be instances where NTNCWSs monitor too much, and TNCWSs do not monitor enough (e.g., extremely high levels of a "chronic" contaminant at a TNCWS may pose an acute risk). Appropriate changes could better utilize limited resources and reduce public health risks. In addition, at least one State participant noted that radionuclides need to be monitored at NTNCWSs.	18	Within the scope of considering for this review effort; best handled through regulatory revision.	Regulatory revisions would be needed to change the applicability of monitoring requirements for NTNCWS and/or TNCWS. See section 2.3 of this document for further discussion.
Chem/Rad Rules *(Radionuclide Rule)*	**Some flexibility on monitoring and reporting should be provided to the extent it does not decrease public health protection. The data and information used to support development of the radionuclide MCLs should also be revisited. The MCLs are too stringent compared to the actual risks posed by radionuclides:** Keeping up with quarterly radium testing is very difficult. By the time samples are collected, analyzed, reported, and data are analyzed, it is nearly impossible to report violations to EPA by the required deadline. Additionally, the way the rule is written, all 4 analytes are required to be	17	Outside the scope of this review effort; the radionuclides rule was effective in 2006 and it is not clear that any changes in monitoring and reporting would clearly improve public health protection; best handled through other mechanisms.	Note that EPA correspondence with commercial labs found that counting time for radiums range from 10 to 300 minutes (depending on radium species and sample volume collected). Regarding MCLs, currently there is no new information to indicate that EPA could consider changes to the MCLs (and it would be considered backsliding to make MCLs less stringent since the MCLG is zero).

Issue[1]	Description	State Workgroup Priority Score	Findings *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
	monitored on possibly different frequencies therefore precluding the ability to substitute the alpha for the more expensive 226 and uranium in routine monitoring.			
Analytical Methods	**Revise performance criteria for the haloacetic acids (HAA5) analytical method:** The HAA5 method specified by 40 CFR 141.131 gives erratic results. When samples are split between separate labs there is little or no correlation between the analytical results. State workgroup members indicated that EPA's own studies showed that many labs could not produce accurate analytical results.	17	Outside the scope of this review effort; best handled through technical assistance (see notes regarding in the Additional Information column).	Experts from EPA's Technical Support Center discussed this issue with the workgroup noting that EPA has not experienced erratic results with the HAA5 method. As part of the quality assurance program for the Information Collection Rule, laboratories were required to perform analyses of fortified samples and report the results to EPA. More than 80 laboratories around the nation participated in that study providing percent recoveries for the 1,250 samples that were fortified for HAA analyses. The data demonstrated that 80% of the HAA recoveries (in fortified field samples) were within 89% and 120% while 80% of the THM recoveries (in fortified field samples) were within 87% and 114%. These data demonstrated that both HAA and THM results were equivalent and that they can both be determined accurately. EPA also provided a contact list of EPA personnel (at TSC and the Office of Research and Development), who are familiar with the analytical methods for various contaminants.

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Lead and Copper Rule	**Amend the LCR to require PWSs that fail to comply with reduced monitoring requirements for lead and copper tap sampling (i.e., annual or tri-annual testing) to return to their initial *monitoring requirements* (6-month testing):** They would then have to complete two consecutive 6-month monitoring periods with results below the action levels before they would again be eligible for reduced monitoring. Currently, PWSs that fail to monitor during a reduced monitoring period could go up to 6 years without having to test for lead and copper.	16	Outside the scope of this review effort since the NPDWR was recently revised and published in 2007.	EPA is currently evaluating issues for the long-term revision effort and this item is best handled by referring it to this effort. In addition, the reduced monitoring issue may be handled best through enforcement actions.
Process Control Measurement	**Clarify and expedite the procedures for approving process control technologies:** Process control technologies often utilize new analytical methods for on-line analyzers and operational testing that are difficult to get accepted. It is difficult to determine what constitutes an acceptable adaptation of a "bench-top" technique. Since they are not analytical methods associated with an MCL, it is uncertain whether the newly proposed, expedited analytical method approval process will help.	16	Outside the scope of this review effort; best handled through technical assistance.	EPA noted during workgroup discussions that online residual analyzers for chlorine would need to go through and be evaluated under the Alternative Test Procedures (ATP) process. EPA provided information and a contact person for EPA's Technical Support Center who could discuss the ATP process for potential evaluation of online analyzers.
Variances and Exemptions Rule	**Streamline and simplify the Variance and Exemption (V & E) Rule:** V & Es could be useful tools if the rule were revised to make them less cumbersome, more	16	Outside the scope of this review effort; best handled through other mechanisms.	The process required for V&Es (other than the small system variances) is specified by the statute (Sections 1415 and 1416). The primacy requirement for States adopting V&E requirements (other than the small system variances)

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	streamlined, and with "less strings attached."			is that they must be no less stringent than the statute. States already have flexibility to adopt whatever process they want as long as it is consistent with the statute (40 CFR 142.10(d)(2)). It is unlikely that EPA can provide any more flexibility on the process for V&Es.
Point of Use and Point of Entry	**Revise Best Available Technologies (BATs) for rules for which point-of-use/point-of-entry (POU/POE) devices may be appropriate, but which are not specifically listed as BATs (e.g., nitrates):** POU/POE are sometimes recognized as BATs but they have not been consistently adopted in existing rules. There may be other situations where this technology could be effectively used as a treatment option and these need to be recognized.	15	Outside the scope of this review effort; best handled through use of existing guidance and technical assistance.	EPA noted that existing guidance includes: (1) "Small System Compliance Technology List for the Non-Microbial Contaminants Regulated before 1996" (EPA 815-R-98-002, September 1998). Available at: www.epa.gov/ogwdw/standard/tretech.html (2) "Point-of-Use Treatment Options for Small Drinking Water Systems". Available at: www.epa.gov/safewater/smallsys/pdfs/guide_smallsystems_pou-poe_june6-2006.pdf. EPA has listed small system POU/POE devices for rules promulgated since the 1996 SDWA Amendments. An August 6, 1998 FR (63 FR 151) notice lists small system devices for rules promulgated before the 1996 SDWA. In addition, a separate discussion was held between EPA staff, ASDWA, and State personnel who were interested in the POU/POE topic. Notes from this meeting are included in Appendix C.
Consumer Confidence Reports	**Revise the Consumer Confidence Report (CCR) to address a number of current implementation issues and concerns, based on experiences in implementing the rule over the past several years:** The amount of system and State resources allocated to the CCR is significant with very limited return. A major	15	Outside the scope of this review effort since CCR is not technically part of an NPDWR.	

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	overhaul of this rule is needed. Possible changes may include that the CCR is done in conjunction with and at the frequency of the sanitary surveys. A second option: The CCR is an EPA report that is maintained by EPA through SDWIS/Fed and is available electronically or in hardcopy from EPA. System owners would be responsible for identifying that the report is available and how to receive a copy.			
False Positive Sample Results	**The regulations should recognize the potential for "false positive" measurement of phthalates and allow any relief from the burden of unnecessary follow-up:** This issue is most common with phthalates, but other contaminants can show up at very low levels (below the practical quantitation limit [PQL]/method reporting limit [MRL]) and trigger additional monitoring and potentially increased levels of treatment when there really is no contamination in the water system but the result is a "false positive".	15	Outside the scope of this review effort; best handled through technical assistance (see notes in the Additional Information column).	EPA's Technical Support Center discussed this item with the workgroup noting compounds such as phthalates can occasionally be observed as false positives. EPA indicated that it is not difficult for laboratories to determine the source of the contamination and eliminate it. The first step is to determine if the contamination is the result of sampling or sample shipment and storage, or if it is a laboratory issue. The next step or solution is to either educate sampling personnel about correct sampling procedures or determine the specific step in the laboratory procedure that needs to be corrected. EPA also noted that the presence of contaminants at or above the MCL needs to be taken seriously. The presumption of a "false positive" may in itself be incorrect, or the "false positive" portion of the result may obscure the actual presence of the analyte in the sample. Using good laboratory and good sampling practices will greatly reduce if not eliminate the problems associated with false positives, even with analytes such as the phthalates.

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				EPA indicated that States, PWSs or laboratories can always call TSC if they are having problems with any EPA-approved methods. EPA provided a contact list of EPA personnel (at TSC and the Office of Research and Development), who are familiar with the analytical methods for various contaminants.

Issue[1]	**Description**	**State Workgroup Priority Score**	**Findings** *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	**Additional Information**
Definition of a public water system	**Revise the definition of a PWS:** Although the definition of a PWS is in the SDWA, it seems that EPA could exercise reasonable flexibility in interpreting the statutory definition to alleviate the confusion between 25 people vs. 15 connections. The definition states, "The term 'public water system' means a system for the provision to the public of water for human consumption through pipes or other constructed conveyances, if such system has at least fifteen service connections or regularly serves at least twenty-five individuals." No preference or guidance is given in the law as to where and when 25 people or 15 connections apply. It would be reasonable, for instance, for EPA to clarify through guidance that "service connections" applies where the PWS actually has individual connections that can be counted (CWSs), and "persons served water" applies to PWSs without individual service connections (TNCWSs, NTNCWSs, businesses, etc.). At least one State participant indicated that it would be useful to clarify what types of consecutive systems are not covered by SDWA, and that this could require a change to section 300g of Act.	14	Outside the scope of this review effort; best handled through guidance (see notes in the Additional Information column).	Current guidance includes Water Supply Guidance memos (#12, 34, 66A, H3 and H18), available at: http://www.epa.gov/safewater/wsg/subject.html#interpretation

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Fluoride	**Revise PN requirements associated with both the MCL and the SMCL to better reflect the implication of violations of each; this may be "folded in" to an overall effort to revise the MCL:** a) The National Academy of Sciences (NAS) has recommended that the MCL be reviewed. b) There is some confusion between the MCL and the SMCL (secondary standard) and the appropriate public notification requirements for each level.	14	Outside the scope of this review effort; best handled through regulatory revision (see notes in the Additional Information column).	While potential MCL changes are within the scope of Six-Year Review, PN alone is technically outside the scope; however, if EPA decides to make changes to the MCL, PN requirements (as well as any issues with the SMCL) will be considered. EPA is currently addressing the NAS recommendations to update the health assessment for fluoride and evaluate exposure sources (relative source contribution). Guidance and assistance could also clarify confusion between MCL and SMCL and the PN requirements in the interim. See section 3.0 of this document for further discussion.
Alternative Treatment	**Allow bottled water to be a compliance technology for appropriate rules and with appropriate caveats:** Some entities may be able to justify the use of bottled water as a viable method of resolving an MCL. Discussion regarding the proposed modification of the bottled water language is requested.	13	Outside the scope of this review effort; best handled through guidance and technical assistance.	At the request of States, EPA held a listening session in December 2006 on the viability of bottled water as an alternative compliance option for chronic contaminants regulated under the SDWA. The meeting aimed to identify what information and data would be needed for EPA to evaluate the efficacy of bottled water as an alternative compliance option for NTNCWSs. In a February 2007 follow up meeting, EPA and ASDWA agreed that current State practices for the use of bottled water on a temporary basis until the system returns to compliance are protective of public health and are being implemented in a responsible manner.

Issue[1]	Description	State Workgroup Priority Score	Findings *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
Chem/Rad Rules *(Nitrate monitoring)*	**Systems with a proven history of nitrate results of less than one-half the MCL (or some other trigger level) should be allowed to reduce nitrate monitoring:** In discussions on Chemical Monitoring Reform several years ago, EPA seemed favorable to this. States have identified many systems with more than 15 years of nitrate data with no nitrate detections. Reducing this monitoring would be helpful and would not decrease protection of public health.	12	Within the scope of considering for this review effort; best handled through regulatory revision.	Regulatory revisions would be needed to change monitoring frequency for these systems. See section 2.2 of this document for further discussion.
Sanitary Surveys	**Clarify applicability of sanitary surveys to various types of systems:** Sanitary survey applicability was expanded to include consecutive systems but not specifically noted in the regulations or special primacy requirements.	11	Outside the scope of this review; effort best handled through other mechanisms.	The requirements for States to conduct sanitary surveys are found in the 40 CFR Section 142,10(b)(2) (general primacy requirement for all PWSs), 142.16(b)(3) (requirement for surface water and GWUDI systems) and 142.16(o)(2)(groundwater systems). The CFR does not list any exclusion for PWSs that are classified as consecutive systems. However, some small subset of consecutive systems may be excluded from all the NPDWRs (including the sanitary survey requirements) under 141.3 (Coverage) and 142.3 (Scope). Public Water Systems excluded from these requirements must meet all of the conditions outlined in those sections including: Consists only of distribution and storage facilities (and does not have any collection and treatment facilities); Obtains all of its water from, but is not owned or operated by, a public water system to which such regulations apply: Does not sell water to any person; and, Is not a carrier which conveys passengers in interstate commerce.

Issue[1]	Description	State Workgroup Priority Score	Findings *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
Underground Injection Control	**Liquid waste generated by a drinking water treatment technology that is recognized by EPA to be a BAT should be exempted from the Underground Injection Control (UIC) requirements or at least establish specific criteria/standards within the revised UIC Rule for all States to adopt for these circumstances:** Some States are interpreting the UIC Class V Rule definition of Sanitary Waste to include "Water Treatment Liquid Waste" (i.e., spent backwash water), thus making it very difficult to secure a discharge permit for this type of liquid waste into on-site septic systems. However, at least one State noted that it did not agree with these exemptions, and their State would classify it as an industrial waste requiring a permit; this State also indicated that the federal standards that apply to the quality of injected waste is pretty specific.	10	Outside the scope of this review effort; best handled through other mechanisms.	
UCMR	**Allow for water system "opt out" from the UCMR for certain specified circumstances:** States are concerned about instances under the UCMR (both UCMR 1 and 2) in which there is no likelihood of a contaminant being present in a water system. That situation is very tough on States that are required by State law to evaluate all SDWA-required laboratory	9	Outside the scope of this review effort; best handled through technical assistance.	Technically, UCMR is not an NPDWR. However, EPA noted that UCMR does allow for State waivers and States can always call EPA if they need clarifications or technical assistance.

Issue[1]	Description	State Workgroup Priority Score	Findings *Within or Outside the Scope of this Six-Year Review Effort* *(Issue best handled through regulatory revision, guidance, technical assistance, other mechanisms)*	Additional Information
	samples for all PWSs under their fee system. Since this situation applies to a few States, it does not get much attention. However, when States are spending resources testing for a contaminant that cannot possibly be there, they have a hard time making the case to elected officials and operators that they should participate in UCMR monitoring.			
VOC detections after painting storage tanks	**VOC detections after painting storage tanks:** Some systems in Texas are having increases in volatile organic compound (VOC) detections due to freshly painted water storage tanks. Minnesota has also had problems with newly installed plastic coatings.	Not scored since this item was added later in workgroup discussions.	Outside the scope of this review effort; best handled through technical assistance.	Regardless of the source, EPA noted during the workgroup meetings that this would still be a detection of a VOC. Systems may need guidance or technical information on how to avoid VOC contamination after a tank has been freshly painted.

1. To compile an initial list of possible issues, the workgroup requested feedback from all States. This nationwide poll resulted in a list of 22 possible implementation-related issues. ASDWA then asked States on the workgroup to rank each of the issues as high, medium, or low priority. Eight workgroup members responded. Total scores used for ranking the issues were calculated by assigning the following values: high priority - 3 points; medium - 2 points; and, low - 1 point. The issues are listed in order of highest to lowest priority score, and their actual score totals are provided in the "Priority Scoring" column.

Appendix C: Summary of the January 18, 2008 Discussion on the Use of POU/POE Devices

Purpose of call: During the most recent conference call (on December 13th) of a state-EPA workgroup that's looking at possible implementation changes for the six year review of regulations, several questions arose related to POU devices. As a follow-up to that discussion, we gathered a few states that have a good deal of experience with POU devices as compliance technologies, along with the EPA-OGWDW experts who developed the Agency's POU guidance to brainstorm a bit more about this. These are the questions/topics to discuss.

- PORTION OF SYSTEM IN COMPLIANCE: What has the Agency said, in guidance and policy, about the percentage of the homes in a community whose homes should be in compliance in order for the system as a whole to be in compliance with the MCL for the contaminant in question? Is there a need to revise what's been said to date? What experiences have states/systems had in this regard that might shed light on this question?
- NITRATE AND POU DEVICES: Can/should POU devices be used for compliance with nitrate? What has the Agency said in this regard in the past? Is there a need for any further clarification of the Agency's intent? (As background on this point, please refer to a letter of March 19, 2001 from Bill Diamond [then with EPA-OGWDW] to EPA Region VII.)
- ROLE OF GUIDANCE VS. REGULATION: As a backdrop to the two questions above, is there any need/value to incorporating any aspects of the response to these questions into regulation?

Attendees: **EPA-OGWDW**: Rajiv Khera, Brian Rourke, Jeff Kempic
Nebraska Drinking Water Program: Jack Daniel
Arizona Drinking Water Program: John Calkins
Texas Drinking Water Program: James Beauchamp
ASDWA: Jim Taft, Darrell Osterhoudt

Percentage of Users that Must Participate:

- **Current Agency Guidance:** Rourke read the portion of the Agency's POU guidance that addresses this issue and noted that: 1) it acknowledges that PWSs may choose to initiate a POU-driven solution to a water quality problem *before* all users have agreed to have POU devices installed; but 2) the guidance encourages PWSs to move expeditiously toward getting all users participating. In addition, the guidance goes on to talk about passing ordinances to cut off a customer's water in the event that the customer will not participate. He explained that the Agency had tried to avoid recommending a particular time frame for getting all users onboard due to concerns that whatever time frame was recommended (e.g., 80 days, 180 days, 270 days, etc.), there would likely have been cases that warranted exceptions to the policy/guidance. He also noted that, if eventual 100% participation is not obtained, the PWS should consider POE devices. Moeller explained that the Drinking Water Protection Division had not spoken to this issue in any of its guidance or training any differently than the Agency's POU guidance does.

- **State Experiences:** Calkins explained that, in his state, the 20 or PWSs that had employed POUs thus far each had 100% customer participation; but, all had been quite small and fairly homogeneous situations. He noted that Arizona's POU guidance allows for start-up of a situation in which POUs are used as a compliance technology if only 75% of the users participate – provided the PWS was moving expeditiously to get all users participating. In such a circumstance, the state would enter into a Consent

Decree with the PWS to require and track their movement toward 100% participation. While no time is set forth in the state's guidance for such incremental progress, he felt that 180 days was about right and any time frame greater than 365 days for getting 100% participation is too long. Daniel said that Nebraska had allowed use of POU devices as compliance technologies for 2 small CWSs and some NTNCs. In each case, 100% participation was required. He expressed the concern that, without a national maximum allowable interval for getting 100% participation, consultants who considering POU devices sometimes tend to play one state off another and seek all of the "wiggle room" allowed by current policy and guidance.

Treating Nitrate with POU Devices:

- **Past and Current Agency Guidance**: Kempic and Rourke explained that the perspective the Agency tried to convey in its POU guidance is that there are POU technologies that will work in removing nitrates, but that none has yet been listed in a rule as a small system compliance technology. They noted that the current caveat in the POU guidance related to nitrate is intended to clearly signal that POUs for nitrate should only be used in those situations where there is a public education component in place that lays out the danger posed by high nitrate levels for at-risk populations and the fact that POU devices typically only protect a single tap. Kempic and Rourke noted that, in the absence of such a component being in place, POU devices for nitrate removal should not be allowed. That caveated prohibition was what was intended by the brief mention of nitrate in Bill Diamond's 2001 guidance memo. They further said that the most appropriate situations for nitrate would be small NTNCs where only adult populations consumed water. Moeller explained that DWPD had developed some guidance that pointed to the attributes of good POU applications that may be helpful in this context.

- **State Experiences**: Calkins noted that there is only one NTNC currently using POU devices for nitrate control in Arizona (at a county park) and another location where approval is pending. He also said that, while he appreciated that POU devices for nitrate posed special concerns, centralized treatment for nitrate is likewise prone to problems and not always reliable. Daniel said that POU devices for nitrate problems are not allowed in his state due to their tendency to fail. He also asked why POU devices were specifically disallowed by the Agency for radon and VOCs. Kempic responded that the principal risk pathway of concern for VOCs and radon was through inhalation, rather than ingestion, thus a POU device at the drinking water tap would not address the volatilized radon or VOCs at the showerhead.

Guidance vs. Regulation

- **Discussion:** The call participants agreed that specific guidance or regulation to further address these questions could be helpful but can also become something of a two-edged sword: on the one hand, it disallows inappropriate uses of POU devices and helps states fend off such uses on the part of water systems or their consultants; on the other hand, prescriptive guidance or regulation can restrict flexibility on the part of states in allowing certain uses and applications that the state deems acceptable. The Agency representatives noted that there is a very "high bar" these days (due to process requirements, Agency manpower needed, etc.) to embarking on a regulatory solution. Changes to guidance (or new guidance) is a less high bar – provided states and EPA, as co-regulators, could agree on revised guidance that seemed to work well for everyone.

- **Next Steps**: It was agreed that a summary of this discussion would be shared with the State-EPA Six Year Implementation Workgroup for their further consideration.